PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, C07K 14/52, 16/24, C12P 21/02

A1

(11) International Publication Number: WO 98/24907

(43) International Publication Date: 11 June 1998 (11.06.98)

(21) International Application Number: PCT/US97/21552

(22) International Filing Date: 26 November 1997 (26.11.97)

(30) Priority Data:
08/760,127 3 December 1996 (03.12.96) US

(71) Applicant: AMGEN INC. [US/US]; Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320–1789 (US).

(72) Inventors: CHANG, Ming–shi; 736 Calle Las Colinas, Newbury Park, CA 91320 (US). ANDREW, David, P.; 4857 Dakota Boulevard, Boulder, CO 80304 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen Inc., Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320–1789 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**

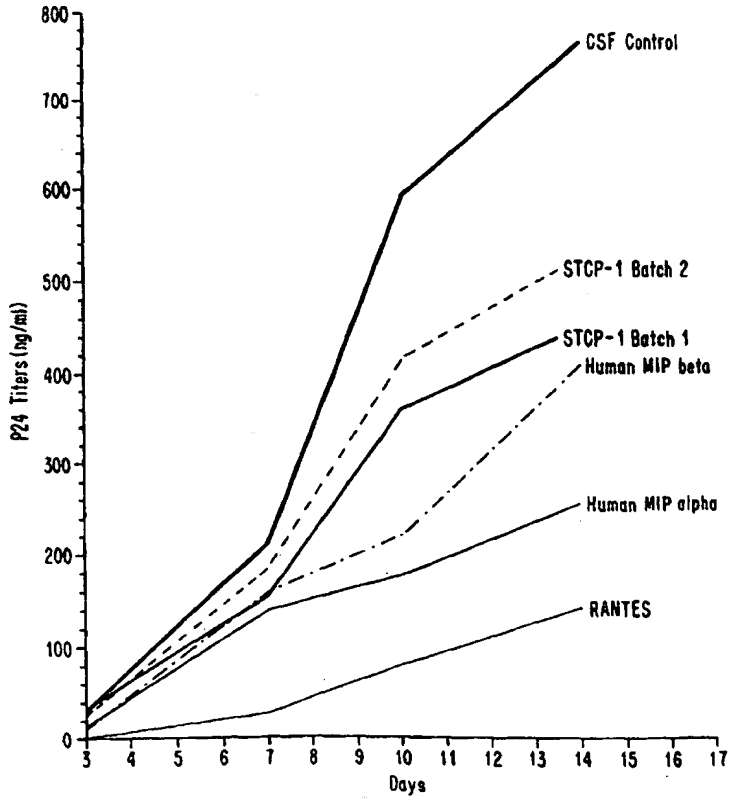
*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: NOVEL PROTEIN WITH CHEMOKINE ACTIVITY

(57) Abstract

Disclosed are nucleic acids encoding a novel chemokine that is specific for T–cells. Also disclosed are the amino acid sequence of the chemokine, and methods for preparing the chemokine.



*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |

# NOVEL PROTEIN WITH CHEMOKINE ACTIVITY

## BACKGROUND

### Field of the Invention

This invention relates to novel polypeptides that have chemokine activity, and to novel nucleic acid molecules encoding such polypeptides.

### Description of Related Art

1. T-cells

The immune system of mammals is comprised of many specialized cells that act together to protect the mammal from invading pathogens, toxins, and other foreign substances.

The cells responsible for the specificity of the immune system response are a class of white blood cells referred to as lymphocytes. Two important sub-classes of lymphocytes are B-cells and T-cells. B-cells exert their effect by producing and secreting antibodies.

There are several types of T-cells, such as helper T-cells (which serve to enhance the activity of other T-cells), suppressor T-cells (which serve to suppress the activity of other white blood cells), and cytotoxic T-cells (which kill other types of cells). Helper T-cells are also referred to as CD4+ T-cells, as they express a protein known as CD4 on their cell surface. Cytotoxic T-cells are also referred to as CD8+ T-cells, as they express CD8 protein on their cell surface.

2. Chemokines

Cytokines are a class of low molecular weight proteins that function in cell-to-cell communication. Chemokines are a subset of cytokines, and serve to recruit leukocytes (white blood cells) into acute and chronic sites of inflammation. There are currently 31 known chemokines (Taub *et al., Therapeutic Immunology*, 1:229-246 [1994]; Furie *et al., Am. J. Pathol.*, 146:1287-1301 [1995]). The chemokines have been further divided into three families based on the position of cysteines in their primary amino acid sequence. These families are referred to as CC, CXC, and Lymphotactin. It has been shown that blocking the activity of certain chemokines may have a profound effect on the inflammatory response. For example, mice deficient in the chemokine MIP-1 alpha have an impaired response to viral infections (Cook *et al., Science*, 269:1583-1585 [1995]).

3. T-cells and HIV

Human Immunodeficiency Virus (HIV), of which there are several primary strains, causes Acquired Immune Deficiency Syndrome (AIDS) in many humans infected with this virus. HIV seems to exert its effect at least in part by invading and destroying CD4+ T-cells. These CD4+ T-cells express the receptor CD4 on their cell surface, and HIV gains entry into the cells in part by binding to CD4. Some strains of HIV (the M-tropic strains) target CD4+ T-cells as well as a class of white blood cells known as macrophages.

Recently, it has been suggested that one or more additional cell surface molecules, or co-receptors, must also be present on the surface of CD4+ T-cells in order for these cells to be infected by HIV.

A recent report by Cocchi *et al.* (*Science*, 270:1811-1815 [1995]) suggests that the known chemokines RANTES, MIP-1 and MIP-2 produced by CD8+ T-cells have HIV-suppressive activity. These findings have, in turn, lead to increased research focused on chemokine receptor molecules (cell surface glycoproteins) and the role such molecules might play in HIV infection. There are currently five known chemokine receptors, which have been designated as CKR-1 to CKR-5. These receptors are members of a family of receptors known as the G-protein coupled receptor family.

Recent reports have suggested that the chemokine receptor CCR-5 may be particularly important for entry of M-trophic HIV strains into target cells (Deng *et al.*, *Nature*, 381:661-666 [1996]; Dragic *et al.*, *Nature*, 381:667-673 [1996]; Choe *et al.*, *Cell*, 85:1135-1148 [1996]), while a separate receptor, called "LESTR" or "fusin", appears to be responsible for entry of T-trophic HIV strains into T-cells (Feng *et al.*, *Science*, 272:872-877 [1996]). The ligand for fusin has recently been identified as the chemokine SDF-1 ("stromal cell derived factor-1"; Bleul *et al.*, *Nature*, 382:829-833 [1996]; Oberlin *et al.*, *Nature*, 382:833-835 [1996]).

It now appears that a certain mutation in CCR-5 can prevent entry of the M-trophic strain of HIV into T-cells, thereby rendering individuals with the CCR-5 mutation potentially "resistant" to M-trophic HIV infection (Hill *et al.*, *Nature*, 382:668-669 [1996]; Samson *et al.*, *Nature*, 382:722-725 [1996]).

At present, there is no prophylactic treatment available for AIDS. Current therapeutic methods of treating HIV infection include administering anti-viral compounds such as AZT, ddC, or ddI, or

combinations thereof, and/or protease inhibitors such as Ritonavir, Invirase and Epivar.

4. T-cells in Immune System Disorders

There are a number of diseases in which T-cells have been directly implicated as causative agents. For example, in autoimmune diseases such as lupus and rheumatoid arthritis, the immune system does not recognize "self" from foreign invaders, and, consequently, destroys the tissues of one's own body. In lupus, multiple sclerosis, and rheumatoid arthritis, for example, T-cells migrate to the affected area (e.g., joints in rheumatoid arthritis), causing inflammation. Antagonists which target T-cells have been shown in some models to be therapeutic (Durie *et al., Science*, 261:1328-1330 [1993]; Lenschow *et al., J. Exp. Med.*, 181:1145-1155 [1995]; Durie *et al., J. Clin. Invest.*, 94:1333-1338 [1994]; Gerritse *et al., Proc. Natl. Acad. Sci. USA*, 93:2499-2504 [1996]; Tak *et al., Arthritis and Rheumatism*, 38:1457-1465 [1995]).

In view of the devastating effects of both HIV infection and AIDS, there is a need in the art to provide novel compounds with anti-HIV activity.

Further, in view of the serious consequences of T-cells in autoimmune diseases and inflammation, there is a need in the art to identify and inhibit the compounds responsible for such T-cell activity.

Accordingly, it is an object of the present invention to provide novel polypeptides with anti-HIV activity.

It is yet a further object to provide nucleic acid molecules encoding such polypeptides and to provide methods of preparing such nucleic acid molecules and polypeptides.

## SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a nucleic acid molecule encoding a polypeptide selected from the group consisting of:

(a) the nucleic acid molecule of SEQ ID NO:1;

(b) the nucleic acid molecule of SEQ ID NO:2;

(c) a nucleic acid molecule encoding the polypeptide of SEQ ID NO:3 or a biologically active fragment thereof;

(d) a nucleic acid molecule that encodes a polypeptide that is at least 70 percent identical to the polypeptide of SEQ ID NO:3;

(e) a nucleic acid molecule that hybridizes under stringent conditions to any of (a)-(d) above; and

(f) a nucleic acid molecule that is the complement of any of (a)-(e) above.

In another embodiment, the invention provides vectors comprising these nucleic acid molecules, and host cells, either prokaryotic or eukaryotic, comprising the vectors.

In yet another embodiment, the invention provides a process for producing a STCP-1 polypeptide, wherein the polypeptide may be SEQ ID NO:3, amino acids 25-93 of SEQ ID NO:3, or a biologically active fragment thereof, and wherein the process comprises:

(a) expressing a polypeptide encoded by a STCP-1 nucleic acid molecule in a suitable host; and

(b) isolating the polypeptide.

The invention further provides a STCP-1 polypeptide selected from the group consisting of:

(a) the polypeptide of SEQ ID NO:3;

(b) the polypeptide that is amino acids 25-93 of SEQ ID NO:3;

(c) a polypeptide that is at least 70 percent identical to the polypeptide of (a) or (b); and

(d) a biologically active fragment of any of (a)-(c). Optionally, the STCP-1 polypeptide may or may not have an amino terminal methionine.

The invention further provides anti-STCP-1 antibodies.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the nucleic acid sequence of the cDNA encoding human STCP-1 (SEQ ID NO:1).

Figures 2A through 2F depict the nucleic acid sequence of the human genomic DNA for STCP-1 (SEQ ID NO:2).

Figure 3 depicts the putative amino acid sequence for human STCP-1 as translated from the cDNA (SEQ ID NO:3). Amino acids 1-24 may represent a signal peptide sequence, such that the mature form of STCP-1 starts at amino acid 25.

Figure 4 depicts a graph of the results of an anti-HIV activity assay for STCP-1. Number of days of incubation of T-cells with HIV strain JR-CSF is indicated on the X-axis, and the P24 antibody titer, a measure of the amount of HIV in the T-cells, is indicated on the Y-axis.

Figure 5(A-D) depicts graphs of the number of cells (X-axis) migrating towards a particular chemokine in a migratory assay. The chemokines in each graph are indicated on the Y-axis. 5A shows the results for TH2 cells previously stimulated once; 5B shows the results for TH2 cells previously stimulated three times; 5C shows the results for TH1 cells previously stimulated three times; and 5D shows the effect of anti-STCP-1

antibody on the migration of TH2 cells previously stimulated three times.

Figure 6 depicts FACScans of chronically activated human T-cells exposed to either MCP-1, eotaxin, or STCP-1 in calcium efflux assays.

Figure 7 depicts FACScans of human eosinophils exposed to either eotaxin, STCP-1, or RANTES.

Figure 8 is a histogram of FACScans. Neutrophils, dendritic cells, and monocytes were exposed to the indicated chemokines using the standard FACScan assay.

## DETAILED DESCRIPTION OF THE INVENTION

Included in the scope of this invention are STCP-1 polypeptides such as the polypeptide of SEQ ID NO:3 and related biologically active polypeptide fragments and derivatives thereof. Further included within the scope of the present invention are nucleic acid molecules that encode these polypeptides, and methods for preparing the polypeptides.

Also included within the scope of the present invention are non-human mammals such as mice, rats, rabbits, goats, or sheep in which the gene (or genes) encoding the human equivalent of STCP-1 has been disrupted ("knocked out") such that the level of expression of this gene is significantly decreased or completely abolished. Such mammals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032. The present invention further includes non-human mammals such as mice, rats,

rabbits, goats, or sheep in which the gene (or genes) encoding the STCP-1 (either the native form of STCP-1 for the mammal or a heterologous STCP-1 gene) is over expressed by the mammal, thereby creating a "transgenic" mammal. Such transgenic mammals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT patent application no. WO94/28122, published 8 December 1994.

The term "STCP-1 protein" or "STCP-1 polypeptide" as used herein refers to any protein or polypeptide having the properties described herein for STCP-1. The STCP-1 polypeptide may or may not have an amino terminal methionine, depending on the manner in which it is prepared. By way of illustration, STCP-1 protein or STCP-1 polypeptide refers to (1) an amino acid sequence encoded by STCP-1 nucleic acid molecules as defined in any of items (a)-(f) below, and peptide or polypeptide fragments derived therefrom, (2) naturally occurring allelic variants of the STCP-1 gene which result in one or more amino acid substitutions, deletions, and/or insertions as compared to the STCP-1 polypeptide of SEQ ID NO:3, and/or (3) chemically modified derivatives as well as nucleic acid and or amino acid sequence variants thereof as provided for herein.

As used herein, the term "STCP-1 fragment" refers to a peptide or polypeptide that is less than the full length amino acid sequence of naturally occurring STCP-1 protein but has substantially the same biological activity as STCP-1 polypeptide or STCP-1 protein described above. Such a fragment may be truncated at the amino terminus, the carboxy terminus, and/or internally, and may be chemically modified. Such STCP-1 fragments may be prepared with or without an amino terminal methionine.

As used herein, the term "STCP-1 derivative" or "STCP-1 variant" refers to a STCP-1 polypeptide, protein, or fragment that 1) has been chemically modified, as for example, by addition of one or more polyethylene glycol molecules, sugars, phosphates, or other such molecules not naturally attached to wild-type STCP-1 polypeptide, and/or 2) contains one or more nucleic acid or amino acid sequence substitutions, deletions, and/or insertions as compared to the STCP-1 amino acid sequence set forth in Figure 3.

As used herein, the terms "biologically active polypeptide" and "biologically active fragment" refer to a peptide or polypeptide in accordance with the above description for STCP-1 wherein the STCP-1 acts as a chemoattractant for activated T-cells.

As used herein, the term "STCP-1" when used to describe a nucleic acid molecule refers to a nucleic acid molecule or fragment thereof that (a) has the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2; (b) has a nucleic acid sequence encoding a polypeptide that is at least 70 percent identical, but may be greater than 70 percent, *i.e.*, 80 percent, 90 percent, or even greater than 90 percent identical, to the polypeptide encoded by any of SEQ ID NOS:1 or 2; (c) is a naturally occurring allelic variant of (a) or (b); (d) is a nucleic acid variant of (a)-(c) produced as provided for herein; (e) has a sequence that is complementary to (a)-(d); and/or (f) hybridizes to any of (a)-(e) under stringent conditions.

Percent sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. By way of example, using a computer program such as BLAST or FASTA, the two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their

respective amino acids (the "matched span", which can include the full length of one or both sequences, or a predetermined portion of one or both sequences). Each computer program provides a "default" opening penalty and a "default" gap penalty, and a scoring matrix such as PAM 250. A standard scoring matrix (see Dayhoff *et al.*, in: *Atlas of Protein Sequence and Structure*, vol. 5, supp.3 [1978]) can be used in conjunction with the computer program. The percent identity can then be calculated using an algorithm contained in a program such as FASTA as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence within the matched span] + [number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Polypeptides that are at least 70 percent identical will typically have one or more amino acid substitutions, deletions, and/or insertions as compared with wild type STCP-1. Usually, the substitutions will be conservative so as to have little or no effect on the overall net charge, polarity, or hydrophobicity of the protein but optionally may increase the activity of STCP-1. Conservative substitutions are set forth in Table I below.

Table I

Conservative amino acid substitutions

| | |
|---|---|
| Basic: | arginine |
| | lysine |
| | histidine |
| Acidic: | glutamic acid |
| | aspartic acid |
| Polar: | glutamine |
| | asparagine |
| Hydrophobic: | leucine |
| | isoleucine |
| | valine |
| Aromatic: | phenylalanine |
| | tryptophan |
| | tyrosine |
| Small: | glycine |
| | alanine |
| | serine |
| | threonine |
| | methionine |

The term "stringent conditions" refers to hybridization and washing under conditions that permit only binding of a nucleic acid molecule such as an oligonucleotide or cDNA molecule probe to highly homologous sequences. One stringent wash solution is 0.015 M NaCl, 0.005 M NaCitrate, and 0.1 percent SDS used at a temperature of 55°C-65°C. Another stringent wash solution is 0.2 X SSC and 0.1 percent SDS used at a temperature of between 50°C-65°C. Where oligonucleotide probes are used to screen cDNA or genomic libraries, the following stringent washing conditions may be used. One protocol uses 6 X SSC with 0.05 percent sodium pyrophosphate at a temperature of 35°C-62°C, depending on the length of the oligonucleotide probe. For example, 14 base pair

probes are washed at 35-40°C, 17 base pair probes are washed at 45-50°C, 20 base pair probes are washed at 52-57°C, and 23 base pair probes are washed at 57-63°C. The temperature can be increased 2-3°C where the background non-specific binding appears high. A second protocol utilizes tetramethylammonium chloride (TMAC) for washing oligonucleotide probes. One stringent washing solution is 3 M TMAC, 50 mM Tris-HCl, pH 8.0, and 0.2 percent SDS. The washing temperature using this solution is a function of the length of the probe. For example, a 17 base pair probe is washed at about 45-50°C.

As used herein, the terms "effective amount" and "therapeutically effective amount" refer to the amount of STCP-1 necessary to support one or more biological activities of STCP-1 as set forth above.

The STCP-1 polypeptides that have use in practicing the present invention may be naturally occurring full length polypeptides, or truncated polypeptides or peptides (*i.e*, "fragments"). The polypeptides or fragments may be chemically modified, *i.e.*, glycosylated, phosphorylated, and/or linked to a polymer, as described below, and they may have an amino terminal methionine, depending on how they are prepared. In addition, the polypeptides or fragments may be variants of the naturally occurring STCP-1 polypeptide (*i.e.*, may contain one or more amino acid deletions, insertions, and/or substitutions as compared with naturally occurring STCP-1).

The full length STCP-1 polypeptide or fragment thereof can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY [1989]) and/or Ausubel *et al.*, eds, (*Current Protocols in Molecular Biology*, Green

Publishers Inc. and Wiley and Sons, NY [1994]). A gene or cDNA encoding the STCP-1 protein or fragment thereof may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Alternatively, a gene encoding the STCP-1 polypeptide or fragment may be prepared by chemical synthesis using methods well known to the skilled artisan such as those described by Engels *et al.* (*Angew. Chem. Intl. Ed.*, 28:716-734 [1989]). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the STCP-1 polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length STCP-1 polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the STCP-1 polypeptide, depending on whether the polypeptide produced in the host cell is secreted from that cell.

In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants of naturally occurring STCP-1. Nucleic acid variants (wherein one or more nucleotides are designed to differ from the wild-type or naturally occurring STCP-1) may be produced using site directed mutagenesis or PCR amplification where the primer(s) have the desired point mutations (see Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra*, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels *et al.*, *supra*, may also be used to

prepare such variants. Other methods known to the skilled artisan may be used as well. Preferred nucleic acid variants are those containing nucleotide substitutions accounting for codon preference in the host cell that is to be used to produce STCP-1. Other preferred variants are those encoding conservative amino acid changes as described above (*e.g.*, wherein the charge or polarity of the naturally occurring amino acid side chain is not altered substantially by substitution with a different amino acid) as compared to wild type, and/or those designed to either generate a novel glycosylation and/or phosphorylation site(s) on STCP-1, or those designed to delete an existing glycosylation and/or phosphorylation site(s) on STCP-1.

The STCP-1 gene or cDNA can be inserted into an appropriate expression vector for expression in a host cell. The vector is selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that amplification of the STCP-1 gene and/or expression of the gene can occur). The STCP-1 polypeptide or fragment thereof may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend at least in part on whether the STCP-1 polypeptide or fragment thereof is to be glycosylated. If so, yeast, insect, or mammalian host cells are preferable; yeast cells will glycosylate the polypeptide, and insect and mammalian cells can glycosylate and/or phosphorylate the polypeptide as it naturally occurs on the STCP-1 polypeptide (*i.e.*, "native" glycosylation and/or phosphorylation).

Typically, the vectors used in any of the host cells will contain 5' flanking sequence (also referred to as a "promoter") and other regulatory elements as well such as an enhancer(s), an origin of

replication element, a transcriptional termination element, a complete intron sequence containing a donor and acceptor splice site, a signal peptide sequence, a ribosome binding site element, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these elements is discussed below. Optionally, the vector may contain a "tag" sequence, *i.e.*, an oligonucleotide sequence located at the 5' or 3' end of the STCP-1 coding sequence that encodes polyHis (such as hexaHis) or another small immunogenic sequence. This tag will be expressed along with the protein, and can serve as an affinity tag for purification of the STCP-1 polypeptide from the host cell. Optionally, the tag can subsequently be removed from the purified STCP-1 polypeptide by various means such as using a selected peptidase for example.

The 5' flanking sequence may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of 5' flanking sequences from more than one source), synthetic, or it may be the native STCP-1 5' flanking sequence. As such, the source of the 5' flanking sequence may be any unicellular prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the 5' flanking sequence is functional in, and can be activated by, the host cell machinery.

The 5' flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, 5' flanking sequences useful herein other than the STCP-1 5' flanking sequence will have been previously identified by mapping and/or by restriction

endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of the 5' flanking sequence may be known. Here, the 5' flanking sequence may be synthesized using the methods described above for nucleic acid synthesis or cloning.

Where all or only a portion of the 5' flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or 5' flanking sequence fragments from the same or another species.

Where the 5' flanking sequence is not known, a fragment of DNA containing a 5' flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion using one or more carefully selected enzymes to isolate the proper DNA fragment. After digestion, the desired fragment may be isolated by agarose gel purification, Qiagen® column or other methods known to the skilled artisan. Selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

The origin of replication element is typically a part of prokaryotic expression vectors purchased commercially, and aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for optimal expression of the STCP-1 polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector.

The transcription termination element is typically located 3' of the end of the STCP-1

polypeptide coding sequence and serves to terminate transcription of the STCP-1 polypeptide. Usually, the transcription termination element in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the element is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described above.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene.

The ribosome binding element, commonly called the Shine-Dalgarno sequence (prokaryotes) or the Kozak sequence (eukaryotes), is necessary for translation initiation of mRNA. The element is typically located 3' to the promoter and 5' to the coding sequence of the STCP-1 polypeptide to be synthesized. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth above and used in a prokaryotic vector.

In those cases where it is desirable for STCP-1 to be secreted from the host cell, a signal sequence may be used to direct the STCP-1 polypeptide out of the host cell where it is synthesized, and the carboxy-terminal part of the protein may be deleted in

order to prevent membrane anchoring. Typically, the signal sequence is positioned in the coding region of STCP-1 nucleic acid sequence, or directly at the 5' end of the STCP-1 coding region. Many signal sequences have been identified, and any of them that are functional in the selected host cell may be used in conjunction with the STCP-1 gene. Therefore, the signal sequence may be homologous or heterologous to the STCP-1 polypeptide, and may be homologous or heterologous to the STCP-1 polypeptide. Additionally, the signal sequence may be chemically synthesized using methods set forth above. In most cases, secretion of the polypeptide from the host cell via the presence of a signal peptide will result in the removal of the amino terminal methionine from the polypeptide.

In many cases, transcription of the STCP-1 polypeptide is increased by the presence of one or more introns on the vector; this is particularly true where STCP-1 is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the STCP-1 nucleic acid sequence, especially where the STCP-1 sequence used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the STCP-1 DNA sequence (as for most cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to the 5' flanking sequence and the STCP-1 coding sequence is important, as the intron must be transcribed to be effective. As such, where the STCP-1 nucleic acid sequence is a cDNA sequence, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably for STCP-1 cDNAs, the intron will be located on one side or the other (*i.e.*, 5' or 3') of the STCP-1 coding sequence such that it does not interrupt the this

coding sequence. Any intron from any source, including any viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

Where one or more of the elements set forth above are not already present in the vector to be used, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the elements are well known to the skilled artisan and are comparable to the methods set forth above (*i.e.*, synthesis of the DNA, library screening, and the like).

The final vectors used to practice this invention are typically constructed from starting vectors such as a commercially available vector. Such vectors may or may not contain some of the elements to be included in the completed vector. If none of the desired elements are present in the starting vector, each element may be individually ligated into the vector by cutting the vector with the appropriate restriction endonuclease(s) such that the ends of the element to be ligated in and the ends of the vector are compatible for ligation. In some cases, it may be necessary to "blunt" the ends to be ligated together in order to obtain a satisfactory ligation. Blunting is accomplished by first filling in "sticky ends" using Klenow DNA polymerase or T4 DNA polymerase in the presence of all four nucleotides. This procedure is well known in the art and is described for example in Sambrook *et al.*, *supra*.

Alternatively, two or more of the elements to be inserted into the vector may first be ligated together (if they are to be positioned adjacent to each other) and then ligated into the vector.

One other method for constructing the vector to conduct all ligations of the various elements simultaneously in one reaction mixture. Here, many nonsense or nonfunctional vectors will be generated due to improper ligation or insertion of the elements, however the functional vector may be identified and selected by restriction endonuclease digestion.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, LaJolla, CA), and pETL (BlueBacII; Invitrogen).

After the vector has been constructed and a STCP-1 nucleic acid has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or STCP-1 polypeptide expression.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when cultured under appropriate conditions, can synthesize STCP-1 protein which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). After collection, the STCP-1 protein can be purified using methods such as molecular sieve chromatography, affinity chromatography, and the like.

Selection of the host cell will depend in part on whether the STCP-1 protein is to be glycosylated or phosphorylated (in which case eukaryotic host cells are preferred), and the manner in which the host cell is able to "fold" the protein into its native tertiary structure (*e.g.*, proper orientation of disulfide bridges, etc.) such that biologically

active protein is prepared by the cell. However, where the host cell does not synthesize biologically active STCP-1, the STCP-1 may be "folded" after synthesis using appropriate chemical conditions as discussed below.

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO) or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, DH5$\alpha$, DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells

may be utilized as host cells in the method of the present invention (Miller *et al.*, *Genetic Engineering* **8**: 277-298 [1986]).

Insertion (also referred to as "transformation" or "transfection") of the vector into the selected host cell may be accomplished using such methods as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

The host cells containing the vector (*i.e.*, transformed or transfected) may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective growth of the transformed cells only is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin.

The amount of STCP-1 polypeptide produced in the host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If the STCP-1 polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. Polypeptides prepared in this way will typically not possess an amino terminal methionine, as it is removed during secretion from the cell. If however, the STCP-1 polypeptide is not secreted from the host cells, it will be present in the cytoplasm (for eukaryotic, gram positive bacteria, and insect host cells) or in the periplasm (for gram negative bacteria host cells) and may have an amino terminal methionine.

For intracellular STCP-1 protein, the host cells are typically first disrupted mechanically or osmotically to release the cytoplasmic contents into a buffered solution. STCP-1 polypeptide can then be isolated from this solution.

Purification of STCP-1 polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (STCP-1/hexaHis) or other small peptide at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (*i.e.*, a monoclonal antibody specifically recognizing STCP-1). For example, polyhistidine binds with great affinity and specificity to nickel, thus an

affinity column of nickel (such as the Qiagen nickel columns) can be used for purification of STCP-1/polyHis. (See for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York [1993]).

Where the STCP-1 polypeptide has no tag and no antibodies are available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined to achieve increased purity. Preferred methods for purification include polyHistidine tagging and ion exchange chromatography in combination with preparative isoelectric focusing.

If it is anticipated that the STCP-1 polypeptide will be found primarily in the periplasmic space of the bacteria or the cytoplasm of eukaryotic cells, the contents of the periplasm or cytoplasm, including inclusion bodies (*e.g.*, gram-negative bacteria) if the processed polypeptide has formed such complexes, can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm by French press, homogenization, and/or sonication. The homogenate can then be centrifuged.

If the STCP-1 polypeptide has formed inclusion bodies in the periplasm, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated with a chaotropic agent

such as guanidine or urea to release, break apart, and solubilize the inclusion bodies. The STCP-1 polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate the STCP-1 polypeptide, isolation may be accomplished using standard methods such as those set forth below and in Marston *et al.* (*Meth. Enz.*, 182:264-275 [1990]).

If STCP-1 polypeptide inclusion bodies are not formed to a significant degree in the periplasm of the host cell, the STCP-1 polypeptide will be found primarily in the supernatant after centrifugation of the cell homogenate, and the STCP-1 polypeptide can be isolated from the supernatant using methods such as those set forth below.

In those situations where it is preferable to partially or completely isolate the STCP-1 polypeptide, purification can be accomplished using standard methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of chromatography (immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

In addition to preparing and purifying STCP-1 polypeptide using recombinant DNA techniques, the STCP-1 polypeptides, fragments, and/or derivatives thereof may be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using methods known in the art such as those set forth by Merrifield *et al.*, (*J. Am. Chem. Soc.*, 85:2149 [1964]), Houghten *et al.* (*Proc Natl Acad. Sci. USA*, 82:5132 [1985]), and Stewart and Young (Solid Phase Peptide Synthesis, Pierce Chem Co, Rockford, IL [1984]). Such polypeptides may be

synthesized with or without a methionine on the amino terminus. Chemically synthesized STCP-1 polypeptides or fragments may be oxidized using methods set forth in these references to form disulfide bridges. The STCP-1 polypeptides or fragments may be employed as biologically active or immunological substitutes for natural, purified STCP-1 polypeptides in therapeutic and immunological processes.

Chemically modified STCP-1 compositions (*i.e.*, "derivatives") where the STCP-1 polypeptide is linked to a polymer ("STCP-1-polymers") are included within the scope of the present invention. The polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. The polymer selected is usually modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled as provided for in the present methods. A preferred reactive aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10 alkoxy or aryloxy derivatives thereof (see U.S. Patent 5,252,714). The polymer may be branched or unbranched. Included within the scope of STCP-1-polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. The water soluble polymer or mixture thereof may be selected from the group consisting of, for example, polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol. For the acylation reactions, the

polymer(s) selected should have a single reactive ester group. For reductive alkylation, the polymer(s) selected should have a single reactive aldehyde group. The polymer may be of any molecular weight, and may be branched or unbranched.

Pegylation of STCP-1 may be carried out by any of the pegylation reactions known in the art, as described for example in the following references: *Focus on Growth Factors* 3: 4-10 (1992); EP 0 154 316; and EP 0 401 384. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described below.

Pegylation by acylation generally involves reacting an active ester derivative of polyethylene glycol (PEG) with an STCP-1 protein. Any known or subsequently discovered reactive PEG molecule may be used to carry out the pegylation of STCP-1. A preferred activated PEG ester is PEG esterified to N-hydroxysuccinimide ("NHS"). As used herein, "acylation" is contemplated to include without limitation the following types of linkages between STCP-1 and a water soluble polymer such as PEG: amide, carbamate, urethane, and the like, as described in *Bioconjugate Chem.* 5: 133-140 (1994). Reaction conditions may be selected from any of those known in the pegylation art or those subsequently developed, provided that conditions such as temperature, solvent, and pH that would inactivate the STCP-1 species to be modified are avoided.

Pegylation by acylation usually results in a poly-pegylated STCP-1 product, wherein the lysine ε-amino groups are pegylated via an acyl linking group. Preferably, the connecting linkage will be an amide. Also preferably, the resulting product will be at least

about 95 percent mono, di- or tri- pegylated. However, some species with higher degrees of pegylation (up to the maximum number of lysine ε-amino acid groups of STCP-1 plus one α-amino group at the amino terminus of STCP-1) will normally be formed in amounts depending on the specific reaction conditions used. If desired, more purified pegylated species may be separated from the mixture, particularly unreacted species, by standard purification techniques, including, among others, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography and electrophoresis.

Pegylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with a protein such as STCP-1 in the presence of a reducing agent. Regardless of the degree of pegylation, the PEG groups are preferably attached to the protein via a $-CH_2-NH-$ group. With particular reference to the $-CH_2-$ group, this type of linkage is referred to herein as an "alkyl" linkage.

Derivatization via reductive alkylation to produce a monopegylated product exploits the differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in STCP-1. Typically, the reaction is performed at a pH (see below) which allows one to take advantage of the $pK_a$ differences between the ε-amino groups of the lysine residues and that of the α-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a water soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled: the conjugation with the polymer occurs predominantly at the N-terminus of the protein without significant modification of other reactive groups such as the

lysine side chain amino groups. The present invention provides for a substantially homogeneous preparation of STCP-1-monopolymer protein conjugate molecules (meaning STCP-1 protein to which a polymer molecule has been attached substantially only (*i.e.*, at least about 95%) in a single location on the STCP-1 protein. More specifically, if polyethylene glycol is used, the present invention also provides for pegylated STCP-1 protein lacking possibly antigenic linking groups, and having the polyethylene glycol molecule directly coupled to the STCP-1 protein.

A particularly preferred water-soluble polymer for use herein is polyethylene glycol, abbreviated PEG. As used herein, polyethylene glycol is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol.

In general, chemical derivatization may be performed under any suitable conditions used to react a biologically active substance with an activated polymer molecule. Methods for preparing pegylated STCP-1 will generally comprise the steps of (a) reacting an STCP-1 polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby STCP-1 becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions will be determined based on known parameters and the desired result. For example, the larger the ratio of PEG: protein, the greater the percentage of poly-pegylated product.

Reductive alkylation to produce a substantially homogeneous population of mono-polymer/STCP-1 protein conjugate molecule will generally comprise the steps of: (a) reacting a STCP-1

protein with a reactive PEG molecule under reductive alkylation conditions, at a pH suitable to permit selective modification of the α-amino group at the amino terminus of said STCP-1 protein; and (b) obtaining the reaction product(s).

For a substantially homogeneous population of mono-polymer/STCP-1 protein conjugate molecules, the reductive alkylation reaction conditions are those which permit the selective attachment of the water soluble polymer moiety to the N-terminus of STCP-1. Such reaction conditions generally provide for $pK_a$ differences between the lysine amino groups and the α-amino group at the N-terminus (the $pK_a$ being the pH at which 50% of the amino groups are protonated and 50% are not). The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired (i.e., the less reactive the N-terminal α-amino group, the more polymer needed to achieve optimal conditions). If the pH is higher, the polymer:protein ratio need not be as large (i.e., more reactive groups are available, so fewer polymer molecules are needed). For purposes of the present invention, the pH will generally fall within the range of 3-9, preferably 3-6.

Another important consideration is the molecular weight of the polymer. In general, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. Similarly, branching of the polymer should be taken into account when optimizing these parameters. Generally, the higher the molecular weight (or the more branches) the higher the polymer:protein ratio. In general, for the pegylation reactions contemplated herein, the preferred average molecular weight is about 2kDa to about 100kDa (the term "about"

indicating ± 1kDa). The preferred average molecular weight is about 5kDa to about 50kDa, particularly preferably about 12kDa to about 25kDa. The ratio of water-soluble polymer to STCP-1 protein will generally range from 1:1 to 100:1, preferably (for polypegylation) 1:1 to 20:1 and (for monopegylation) 1:1 to 5:1.

Using the conditions indicated above, reductive alkylation will provide for selective attachment of the polymer to any STCP-1 protein having an α-amino group at the amino terminus, and provide for a substantially homogenous preparation of monopolymer/STCP-1 protein conjugate. The term "monopolymer/STCP-1 protein conjugate" is used here to mean a composition comprised of a single polymer molecule attached to an STCP-1 protein molecule. The monopolymer/STCP-1 protein conjugate preferably will have a polymer molecule located at the N-terminus, but not on lysine amino side groups. The preparation will preferably be greater than 90% monopolymer/STCP-1 protein conjugate, and more preferably greater than 95% monopolymer STCP-1 protein conjugate, with the remainder of observable molecules being unreacted (i.e., protein lacking the polymer moiety). The examples below provide for a preparation which is at least about 90% monopolymer/ protein conjugate, and about 10% unreacted protein. The monopolymer/protein conjugate has biological activity.

For the present reductive alkylation, the reducing agent should be stable in aqueous solution and preferably be able to reduce only the Schiff base formed in the initial process of reductive alkylation. Preferred reducing agents may be selected from the group consisting of sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine

borane and pyridine borane. A particularly preferred reducing agent is sodium cyanoborohydride.

Other reaction parameters, such as solvent, reaction times, temperatures, etc., and means of purification of products, can be determined based on the published information relating to derivatization of proteins with water soluble polymers.

A mixture of polymer-STCP-1 protein conjugate molecules may be prepared by acylation and/or alkylation methods, as described above, and one may select the proportion of monopolymer/ protein conjugate to include in the mixture. Thus, where desired, a mixture of various protein with various numbers of polymer molecules attached (i.e., di-, tri-, tetra-, etc.) may be prepared and combined with the monopolymer/STCP-1 protein conjugate material prepared using the present methods.

Generally, conditions which may be alleviated or modulated by administration of the present polymer/STCP-1 include those described herein for STCP-1 molecules in general. However, the polymer/STCP-1 molecules disclosed herein may have additional activities, enhanced or reduced activities, or other characteristics, as compared to the non-derivatized molecules.

STCP-1 nucleic acid molecules, fragments, and/or derivatives that do not themselves encode polypeptides that are active in activity assays may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of STCP-1 DNA or RNA in mammalian tissue or bodily fluid samples.

STCP-1 polypeptide fragments and/or derivatives that are not themselves active in activity assays may be useful as modulators (e.g., inhibitors or

stimulants) of the STCP-1 receptors *in vitro* or *in vivo*, or to prepare antibodies to STCP-1 polypeptides.

The STCP-1 polypeptides and fragments thereof, whether or not chemically modified, may be employed alone, or in combination with other pharmaceutical compositions such as, for example, AZT, ddI, ddC, protease inhibitors, other cytokines, interferons, interleukins, growth factors, antibiotics, anti-fungal compounds, and/or anti-inflammatories.

The STCP-1 polypeptides and/or fragments thereof may be used to prepare antibodies generated by standard methods. Thus, antibodies that react with the STCP-1 polypeptides, as well as reactive fragments of such antibodies, are also contemplated as within the scope of the present invention. The antibodies may be polyclonal, monoclonal, recombinant, chimeric, single-chain and/or bispecific. Typically, the antibody or fragment thereof will be "humanized", *i.e.*, prepared so as to prevent or minimize an immune reaction to the antibody when administered to a patient. The antibody fragment may be any fragment that is reactive with the STCP-1 of the present invention, such as, $F_{ab}$, $F_{ab'}$, etc. Also provided by this invention are the hybridomas generated by presenting STCP-1 or a fragment thereof as an antigen to a selected mammal, followed by fusing cells (*e.g.*, spleen cells) of the mammal with certain cancer cells to create immortalized cell lines by known techniques. The methods employed to generate such cell lines and antibodies directed against all or portions of a human STCP-1 polypeptide of the present invention are also encompassed by this invention.

The antibodies may be used therapeutically, such as to inhibit binding of STCP-1 to its receptor. The antibodies may further be used for *in vivo* and *in*

*vitro* diagnostic purposes, such as in labeled form to detect the presence of the STCP-1 in a body fluid.

Therapeutic Compositions and Administration

Therapeutic compositions for treating HIV infection, AIDS, and related disorders or diseases are within the scope of the present invention. Such compositions may comprise a therapeutically effective amount of a STCP-1 polypeptide or fragment thereof (either of which may be chemically modified) in admixture with a pharmaceutically acceptable carrier. The carrier material may be water for injection, preferably supplemented with other materials common in solutions for administration to mammals. Typically, a STCP-1 therapeutic compound will be administered in the form of a composition comprising purified STCP-1 polypeptide or fragment (which may be chemically modified) in conjunction with one or more physiologically acceptable carriers, excipients, or diluents. Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. Preferably, the product is formulated as a lyophilizate using appropriate excipients (*e.g.*, sucrose). Other standard carriers, diluents, and excipients may be included as desired. Other exemplary compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

The STCP-1 compositions can be systemically administered parenterally. Alternatively, the compositions may be administered intravenously or subcutaneously. When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such pharmaceutically acceptable protein solutions, with due

regard to pH, isotonicity, stability and the like, is within the skill of the art.

Therapeutic formulations of STCP-1 compositions useful for practicing the present invention may be prepared for storage by mixing the selected composition having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, 18th edition, A.R. Gennaro, ed., Mack Publishing Company [1990]) in the form of a lyophilized cake or an aqueous solution. Acceptable carriers, excipients or stabilizers are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The STCP-1 composition to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. Where the STCP-1 composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration ordinarily will be stored in lyophilized form or in solution.

Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration of the composition is in accord with known methods, *e.g.* oral, injection or infusion by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained release systems or implantation device which may optionally involve the use of a catheter. Where desired, the compositions may be administered continuously by infusion, bolus injection or by implantation device. Alternatively or additionally, STCP-1 may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which STCP-1 polypeptide has been absorbed.

Where an implantation device is used, the device may be implanted into any suitable tissue or organ, such as, for example, into a cerebral ventricle or into brain parenchyma, and delivery of STCP-1 may be directly through the device via bolus or continuous administration, or via a catheter using continuous infusion.

STCP-1 polypeptide may be administered in a sustained release formulation or preparation. Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamine (Sidman *et al*, *Biopolymers*, 22: 547-556 [1983]), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J.*

*Biomed. Mater. Res.*, 15: 167-277 [1981] and Langer, Chem. Tech., 12: 98-105 [1982]), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art (*e.g.*, DE 3,218,121; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 [1985]; Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 4030-4034 [1980]; EP 52,322; EP 36,676; EP 88,046; EP 143,949).

In some cases, it may be desirable to use STCP-1 compositions in an *ex vivo* manner, *i.e.*, to treat cells or tissues that have been removed from the patient and are then subsequently implanted back into the patient.

In other cases, STCP-1 may be delivered through implanting into patients certain cells that have been genetically engineered to express and secrete STCP-1 polypeptide. Such cells may be animal or human cells, and may be derived from the patient's own tissue or from another source, either human or non-human. Optionally, the cells may be immortalized. The cells may be implanted into suitable body tissues or organs of the patient.

An effective amount of the STCP-1 composition(s) to be employed therapeutically will depend, for example, upon the therapeutic objectives such as the indication for which STCP-1 is being used, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage may range from about 0.1 μg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, a

clinician will administer the STCP-1 composition until a dosage is reached that achieves the desired effect. The STCP-1 composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of STCP-1) over time, or as a continuous infusion via implantation device or catheter.

As further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, the type of disorder under treatment, the age and general health of the recipient, will be able to ascertain proper dosing.

STCP-1 protein and/or fragments or derivatives thereof, may be used as a prophylactic or therapeutic treatment for HIV infection. Further, STCP-1 protein and/or fragments or derivatives thereof may be used as a therapeutic or prophylactic treatment for other viral or bacterial pathogens which infect T-cells, macrophages and/or other immune system cells, and which cells can be at least partially protected from infection by administration of STCP-1.

Alternatively, or additionally, STCP-1 levels in the cirulatory system may be inhibited by a suitable drug or drugs in order to alleviate symptoms such as joint inflammation associated with rheumatoid arthritis, lupus, or other autoimmune disease.

In certain situations, it may be desirable to use gene therapy methods for administration of STCP-1 to patients suffering from HIV infection, AIDS, or other diseases for which STCP-1 is a viable therapeutic agent. In these situations, genomic DNA, cDNA, and/or synthetic DNA encoding STCP-1 or a fragment or variant thereof may be operably linked to a constitutive or inducible promoter (where the promoter may be

homologous or heterologous) that is active in the tissue into which the composition will be injected. This construct can then be inserted into a suitable vector such as an adenovirus vector or a retrovirus vector to create a "gene therapy vector". The cells of the patient to be treated can be removed from the patient, infected with the gene therapy vector using standard transfection procedures for eukaryotic cells, and tested for STCP-1 protein production. Those cells expressing STCP-1 can then be re-introduced into the patient.

Gene therapy methods may also be employed where it is desirable to inhibit STCP-1 activity. Here, antisense DNA or RNA with a sequence that is complementary to at least the portion of the gene encoding STCP-1 can be prepared, placed into a suitable vector, and transfected into selected cells (previously removed from the patient in an *ex vivo* manner). The vector is typically selected based on its ability to generate high levels of the anti-sense RNA in conjunction with the host cell's machinery.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of STCP-1. In this situation, the DNA encoding a mutant full length or truncated polypeptide of STCP-1 is inserted into a retrovirus or adenovirus, or a comparable vector, and the vector in turn is transfected into the patient's cells in either an *ex vivo* or *in vivo* manner. This STCP-1 mutant is designed to compete with endogenous STCP-1 in binding to the STCP-1 receptor.

Assays to Screen for Inhibitors of STCP-1

As mentioned above, it may, in some situations, be desirable to inhibit or significantly decrease the level of STCP-1 activity. Compounds that

inhibit STCP-1 activity could be administered either in an *ex vivo* manner, or in an *in vivo* manner by local or iv injection, or by oral delivery, implantation device, or the like. The assays described below provide examples of methods useful for identifying compounds that could inhibit STCP-1 activity.

For ease of reading, the following definition is used herein for describing the assays:

"Test molecule(s)" refers to the molecule(s) that is under evaluation as an inhibitor of STCP-1, typically by virtue of its potential ability to block the interaction of STCP-1 with its receptor.

Several types of *in vitro* assays using purified protein may be conducted to identify those compounds that disrupt STCP-1 activity. Such disruption may be accomplished by a compound that typically inhibits the interaction of STCP-1 with its receptor.

In one assay, purified STCP-1 protein or a fragment thereof (prepared for example using methods described above) can be immobilized by attachment to the bottom of the wells of a microtiter plate. Radiolabeled STCP-1 receptor, as well as the test molecule(s) can then be added either one at a time or simultaneously to the wells. After incubation, the wells can be washed and counted using a scintillation counter for radioactivity to determine the degree of STCP-1/receptor binding in the presence of the test molecule. Typically, the molecule will be tested over a range of concentrations, and a series of control "wells" lacking one or more elements of the test assays can be used for accuracy in evaluating the results. A variation of this assay involves attaching the receptor to the wells, and adding radiolabeled STCP-1 along with the test molecule to the wells. After incubation and washing, the wells can be counted for radioactivity.

Several means other than radiolabelling are available to "mark" STCP-1. For example, STCP-1 protein can be radiolabelled using 125-I. Alternatively, a fusion protein of STCP-1 wherein the DNA encoding STCP-1 is fused to the coding sequence of a peptide such as the *c-myc* epitope. STCP-1-myc fusion protein can readily be detected with commercially available antibodies directed against *myc*.

An alternative to microtiter plate type of binding assays comprises immobilizing either STCP-1 or its receptor on agarose beads, acrylic beads or other types of such inert substrates. The inert substrate containing the STCP-1 or its receptor can be placed in a solution containing the test molecule along with the complementary component (either receptor or STCP-1 protein) which has been radiolabeled or fluorescently labeled; after incubation, the inert substrate can be precipitated by centrifugation, and the amount of binding between STCP-1 and receptor can be assessed using the methods described above. Alternatively, the insert substrate complex can be immobilized in a column and the test molecule and complementary component passed over the column. Formation of the STCP-1/receptor complex can then be assessed using any of the techniques set forth above, *i.e.*, radiolabeling, antibody binding, or the like.

Another type of *in vitro* assay that is useful for identifying a molecule to inhibit STCP-1 activity is the Biacore assay system (Pharmacia, Piscataway, NJ) using a surface plasmon resonance detector system and following the manufacturer's protocol. This assay essentially involves covalent binding of either STCP-1 or its receptor to a dextran-coated sensor chip which is located in a detector. The test molecule and the complementary component can then be injected into the chamber containing the sensor chip either

simultaneously or sequentially, and the amount of binding of STCP-1/receptor can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test molecules together for use in decreasing or inhibiting STCP-1 activity. In these cases, the assays set forth above can be readily modified by adding such additional test molecule(s) either simultaneously with, or subsequently to, the first test molecule. The remainder of steps in the assay can be as set forth above.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

## EXAMPLES

Standard methods for library preparation, DNA cloning, and protein expression are set forth in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY [1989]) and in Ausubel *et al*, eds. (*Current Protocols in Molecular Biology*, Wiley, New York, NY [1995]).

### Example I: Cloning of DNA Encoding STCP-1

Human macrophages were used as the tissue source for preparing a cDNA library. Macrophages were generated by *in vitro* differentiation of monocytes, and the monocytes were prepared as follows. Human blood

was subjected to Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density separation using standard procedures. Monocytes were collected after centrifugation, pelleted, and resuspended at a density of about 5,000,000 per ml in Dulbecco's Modified Eagle's Medium (DMEM) containing about two percent fetal calf serum. About eighty ml of cell suspension was plated into each of ten 150 cc Falcon culture flasks (Becton Dickinson, Franklin Lakes, NJ) and the flasks were incubated for about two hours, after which time the nonadherent cells (monocytes adhered) were removed by two washes with warm medium. Each flask then received about 40 ml of RPMI 1640 medium containing 10 percent human serum type AB (Gemini Bioproducts, Calabasas, CA), 5 percent fetal calf serum, 50 mM calcitriol (Biomol, Plymouth Meeting, PA), 292 µg/ml L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. The flasks were incubated at 37°C in an incubator with 10 percent carbon dioxide. The medium was changed on days 3 and 6. On day 7, the medium of each flask was adjusted to contain one of the following stimuli: 1 µg/ml lippopolysaccharide ("LPS" from *E coli* K562; Sigma, St. Louis, MO) 100 U/ml human recombinant interferon-gamma (R&D Systems, Minneapolis, MN); 1 µg/ml LPS plus 100 U/ml human recombinant interferon-gamma; 100 ng/ml human recombinant tumor necrosis factor plus 10 ng/ml human recombinant IL-1b (both, R&D Systems, Minneapolis, MN); 50 ng/ml human recombinant IL-4 (R&D Systems, Minneapolis, MN); 10 ng/ml human recombinant IL-10 (R&D Systems, Minneapolis, MN); or 10 ng/ml human recombinant consensus-interferon (Amgen, Thousand Oaks, CA). The cells were incubated for three hours after which time the medium was removed and ice cold PBS containing 5 mM EDTA was added, and all cells were then combined.

The mRNA from the cells was isolated using the Invitrogen Fast Track mRNA isolation kit

(Invitrogen, San Diego, CA) and following the manufacturer's protocol.

Using this mRNA, a cDNA library was prepared as follows: About five micrograms of mRNA was used to prepare poly(A)-RNA by following the Invitrogen manufacturer's protocol. This poly(A)-RNA was random primed using standard hexamer primers and reverse transcribed to make single-stranded cDNA using reverse transcriptase. Double stranded cDNA was then prepared and subcloned into the vector pcDNAII (Invitrogen) using a cDNA Copy Kit (Invitrogen, San Diego, CA). The average size of the cDNAs in the library was about 1 kb (kilobase), as determined by agarose gel electrophoresis.

About two thousand clones from this library were randomly selected, and their DNA was isolated using standard minipreparation procedures (see Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY [1989]). For each of these clones, about 300 base pairs (bp) of DNA was sequenced from both the 3' and 5' ends of each clone. These sequences were then compared with known DNA sequences using the computer program FASTA to assess homology of the clones to the known sequences. One clone, fhm-00003-a6, had about thirty five percent homology at the translated amino acid sequence level with the chemokine RANTES. The 5' sequence for fhm-00003-a6 was obtained by polymerase chain reaction (PCR). The template for this reaction was the pool of cDNA inserts of the total macrophage library in the pcDNAII vectors. Primers specific for a 5' region of fhm-00003-a6 and the universal primer site of the vector were used for PCR. The conditions for PCR were: 94°C for 15 seconds; 60°C for 15 seconds; and 72°C for 30 seconds. Twenty-five cycles of PCR were conducted.

An amplification product of about 150 base pairs was obtained by this PCR. This product was sequenced and found to have an amino terminal methionine.

To obtain the full length cDNA, PCR primers directed to the 5' end of fhm-00003-a6 and the 3' end of the coding sequence were prepared. The template for PCR was the pool of cDNA inserts of the total macrophage library in the pcDNAII vectors. PCR conditions were: 94°C for 15 seconds; 60°C for 15 seconds; and 72°C for 30 seconds. Twenty-five cycles of PCR were conducted. An amplification product of about 282 base pairs, encoding full length STCP-1, was obtained and sequenced.

The sequence of this full length cDNA is shown in Figure 1 (SEQ ID NO:1). The predicted amino acid sequence of the polypeptide corresponding to this cDNA is shown in Figure 3 (SEQ ID NO:3). The full length amino acid sequence is 93 amino acids. There is a putative signal sequence spanning amino acids 1-24; the mature (secreted) polypeptide therefore is likely to be amino acids 25-93.

The genomic DNA of STCP-1 was obtained from a human genomic P1 library (Genome Systems Inc., St. Louis, MO; catalog no.P1-2535). The library was screened using the STCP-1 cDNA as a probe. The cDNA was radiolabeled using the Amersham Rediprime kit (Amersham, Arlington Heights, IL; catalog no. RPN-1633) and the hybridization and prehybridization solution was: 50 percent formamide, 5 X SSC, 5 X Denhardt's, 0.05 percent sodium pyrophosphate, 0.1 percent SDS, and 100 µg/ml salmon sperm DNA. Prehybridization was for about 1 hour, and hybridization was for about 16 hours at 42°C.

After hybridization, the filters were washed in 0.2 X SSC and 0.1 percent SDS at 42°C for about 1

hour, and then exposed to film. Two positive clones were identified, and the plasmids containing these clones were purified according to Genome Systems Inc. protocols. The plasmid DNA was then sequenced directly.

The genomic sequence of STCP-1 is shown in Figure 2 (SEQ ID NO:2). The gene consists of 3 exons and 2 introns.

Example II: Preparation of STCP-1 Protein

A cDNA clone of STCP-1 coding amino acids 25-93 was inserted into the vector pAMG21 which is a derivative of pCFM 1656 (ATCC accession number 69576) and contains appropriate restriction sites for insertion of genes downstream from the *lux* PR promoter (see US Patent No. 5,169,318 for a description of the *lux* expression system). The host cell used was *E coli* K12, strain CGSC 6159 (Yale University genetic stock, New Haven, CT). The host cells were transformed with the vector using standard transformation procedures, and were then incubated in 2XYT medium containing about 50 µg/ml kanamycin at 30°C. Induction of STCP-1 gene product was commenced by adding the autoinducer N-(3-oxohexanoyl)-DL-homoserine lactone to the culture medium to a final concentration of about 30 ng/ml, and the cultures were incubated at either 30°C or 37°C for about 6 hours after which time the cells were examined by microscopy for inclusion bodies.

The majority of STCP-1 protein was found to be located in the inclusion bodies. Therefore, a cell paste was prepared by pelleting the cells. To extract protein from the inclusion bodies, the cell paste was placed into a solubilization solution containing about 7 M guanidine and 7.5 mM DTT. The solubilized protein was then eluted into a second buffer containing about 4

M urea, and was then passed over a cation exchange column followed by a hydrophobic interaction column. The protein was dialyzed before loading a sample on to SDS-PAGE to assess purity. Coomassie staining of the gel indicated that the protein was at least 95 percent pure.

Example III: Preparation of STCP-1 Antibodies

Rabbit polyclonal anti-STCP-1 antibodies were prepared by coupling about 1 mg of STCP-1 (the mature form encoding amino acids 25-93)to about 1 mg of maleimide activated KLH (Pierce Chemical Co., Rockford, IL) by adding the two components to about 2 ml of Imject Maleimide Buffer (Pierce Chemical Co., Rockford, IL) for about 1 hour at room temperature and then passing the solution over a desalting column (Pierce Chemical Co., Rockford, IL) and collecting the relevant fractions. The STCP-1/KLH complex containing fractions were collected and the concentration was adjusted to about 1 mg/ml. This solution was then combined with an equal volume of Titermax (CytRx Corporation, Norcross, GA) and the mixture was emulsified by passage through a 20 gauge needle.

Three rabbits were immunized at three positions on their backs with about 0.1 ml of the Titermax:STCP-1/KLH mixture on days 0, 30, and 44. About one week after the last immunization, the three rabbits were bled and the serum was collected and pooled. Rabbit immunoglobulin was then purified by Protein A affinity chromatography, and this purified immunoglobulin was tested by ELISA for binding to STCP-1. The antiserum was used at a concentration of about 20 µg/ml as described below in the calcium flux assays.

Example IV: STCP-1 Activity Assays

1. Anti-HIV Activity

Human peripheral white blood cells were cultured in RPMI medium (Gibco/BRL, Grand Island, NY). To stimulate the cells, PMA (Sigma Chemical Co., St. Louis, MO) was added at a concentration of about 5 µg/m, and the cells were incubated for about 48 hours. After incubation, the cells were washed with RPMI medium, and then fresh RPMI medium containing human recombinant IL-2 (Amgen, Thousand Oaks, CA) at a concentration of about 10U/ml and 20 percent fetal calf serum was added to the cells.

The cells were infected with either the JR-CSF (T-cell specific) or the NL-4-3 strain (macrophage specific) of HIV by adding about 100,000 cells to each well of a 24-well Petri dish followed by about 0.5 ng of virus (as measured by p24 antigen concentration on the virus). The cells were incubated at 37°C in an incubator for about 2 hours. After infection, the cells were washed with serum free medium RPMI plus 100 U/ml IL-2, and then placed in RPMI medium containing 100 U/ml IL-2 plus 20 percent fetal calf serum in the presence of about 500 ng/ml of human recombinant (hr) RANTES, 500 ng/ml hrMIP-1, 500 ng/ml hrMIP-2, or 500 ng/ml STCP-1 (mature form, amino acids 25-93). Aliquots of cells were removed at 3, 7, 10, and 14 days after infection. Viral infection of the cells was determined by ELISA assay using the antibody p24 and the ELISA kit (Colter Inc.; catalog no. PN6603662). These experiments were repeated twice using separate batches of STCP-1 protein.

The results of the JR-CSF virus infection are shown in Figure 4. Days after infection is indicated on the X axis, while p24 antibody titer, a measure of infection of the cells, is shown on the Y axis. As can

be seen, RANTES significantly decreased the number of infected cells, and STCP-1 was also effective at reducing the number of infected cells.

2. Chemokine Activity of STCP-1

A. Preparation of TH1 and TH2 Cells

About two ml of PBS (phosphate buffered saline) containing about 1 mM MgCl2, 1 mM CaCl2, 4 µg/ml of anti-CD3 monoclonal antibody from OKT3 hybridoma cells (obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, USA; the antibody was obtained by culturing the cells, collecting the supernatant, and passing it through a protein G affinity column [Pharmacia]), and 10 µg/ml anti-CD28 monoclonal antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) was added to each well of a 6 well Falcon tissue culture plate (38 mm wells). This mixture was incubated about 6 hours at 37°C in a carbon dioxide incubator. The wells of each plate were then washed three times with about 3 ml of PBS per wash.

CD4+ T-cells from human umbilical cord tissue were obtained from Poietic Technologies (Germantown, MD). These cells were divided into two groups and resuspended to a concentration of about 100,000 cells per ml in either TH1 or TH2 medium, and about 3 ml of cell suspension of TH1 or TH2 was then added per well in the 6 well Falcon plates previously coated with anti-CD3 and anti-CD28 as described above.

Both the TH1 and TH2 media contained the following ingredients: DMEM (Gibco/BRL, Grand Island, NY); 10 percent fetal calf serum (Hyclone Labs, Logan, Utah), penicillin at about 1000 U/ml, streptomycin at about 1000 U/ml (Gibco/BRL), glutamine at about 290 µg/ml, mercaptoethanol at about 50 µM. non-essential

amino acids (Gibco/BRL) sodium pyruvate at about 100 µM (Gibco/BRL), and 10 mM Hepes. In addition, TH1 medium contained the following components: human recombinant (hr) IL-1, hrIL-2, and hrIL-6 at about 100 U/ml, and hrIL-12 at about 0.5 ng/ml. TH2 medium additionally contained the following components: hrIL-1, hrIL-2 and hrIL-6 at about 100 U/ml, and hrIL-4 at about 0.5 ng/ml. All interleukins were obtained from R and D Systems (Minneapolis, MN)

The cells in the Falcon plates were incubated in TH1 or TH2 medium for about 7 days at 37°C in a carbon dioxide incubator. After the incubation period, the cells were washed twice in DMEM medium and cultured for an additional 7 days in the following medium: DMEM (Gibco/BRL, Grand Island, NY); 10 percent fetal calf serum (Hyclone Labs, Logan, Utah), penicillin at about 1000 U/ml, streptomycin at about 1000 U/ml (Gibco/BRL), glutamine at about 290 µg/ml, mercaptoethanol at about 50 µM. non-essential amino acids (Gibco/BRL) sodium pyruvate at about 100 µM (Gibco/BRL), 10 mM Hepes, and about 400 U/ml hrIL-2 (R and D Systems, Minneapolis, MN).

In some cases, the cycle of stimulating the cells by washing the cells in PBS, followed by activating them on anti-CD28 and anti-CD3 coated plates, followed by incubating them in the TH1 or TH2 medium containing interleukins (see above) was repeated one or two times to yield cells that had been stimulated a total of either one, two, or three times.

After all cycles of stimulation and incubation, the cells were pelleted by centrifugation, and resuspended in RPMI medium (Gibco/BRL, Grand Island, NY) containing about 0.5 percent human serum albumin (Armour Pharmaceutical Company, Kankakee, IL) and 10 mM Hepes at a density of about 4 x 10(6) cells/

ml of medium. These cells were then used in the chemoattractant assay.

B. Preparation of Monocytes

About 60-70 ml of human blood was obtained in EDTA Vacutainers (Beckton-Dickinson, Rutherford, NY; catalog no. 07070), diluted 1:2 (v:v) into sterile PBS, and layered on to 1-Step Polymorphs (Accurate Inc. Westbury, NY; catalog no. AN 221,710). The 1-Step Polymorph preparation was centrifuged at 450-500 X g for about 30 minutes at 20°C. The mononuclear cell layer, which was the top layer, was then collected, as was the lower band (which contained neutrophils and eosinophils). All cell samples were then diluted to about 50 ml with PBS, and spun in conical tubes at 780 X g for about 15 minutes.

After spinning, the pellets were placed on ice and about 15 ml of E-Lyse (Cardinal Associates, Santa Fe, NM) was added to each pellet. The pellets were incubated about 10 minutes after which they were washed three times with ice cold PBS and spun at about 200 X g.

The purified mononuclear cells were found to contain NK cells, B cells, and T cells as well as monocytes and a minor population of dendritic cells. These cells were used in the calcium flux and chemotaxis assays (see below) as a source of monocytes by using the FACScan to gate on the monocytes at the end of each experiment based on the unique size and scatter properties of monocytes.

C. Preparation of Eosinophils

Eosinophils were obtained as described immediately above. After washing, the cells were then resuspended at a density of about 10,000,000 cells/ml in ice cold DMEM containing about 2 percent fetal calf

serum. Mouse anti-CD16 antibody (Immunotech, Westbrook, ME; catalog no. 0813) was then added to the cell suspension at 10-20 µg/ml, and the cells were rotated at 4°C for 30-60 minutes, after which time the cells were again washed three times in ice cold DMEM.

To remove any neutrophils from the preparation, sheep anti-mouse IgG Dynabeads (Dynal, Lake Services, NY) were added to the cells in an approximate amount of 4 beads per cell (the beads come at a concentration of about 400,000 beads/µl). Prior to adding the beads to the cells, the beads were washed twice with ice cold PBS, and twice with ice cold DMEM containing about 2 percent fetal calf serum. The cells and beads were combined in a total volume of about 10 ml DMEM containing two percent fetal calf serum and mixed by rotation at 4°C for 30-60 minutes. The contents of the mixture was then divided into two five-ml aliquots, each of which was placed on a magnet. After five minutes, the unbound cells (which were the eosinophils) were removed and combined into a 15 ml conical tube. These cells were then spun in a clinical centrifuge for 10 minutes at about 1500 rpm. The pellet was then resuspended in about 5 ml of ice cold PBS, and the cells were counted.

To assess the purity of the eosinophil preparation, about 100,000-250,000 cells were used for a cytospin in a maximum volume of about 150 µl. The cells were spun on the slide for about 3 minutes at 300 rpm, fixed with methanol, and stained with Diff-Quick. This procedure provided an eosinophil population that was greater than 95 percent pure.

D. Preparation of Dendritic Cells

About 60-70 ml of human blood was obtained in EDTA Vacutainers (Beckton-Dickinson, Rutherford, NY; catalog no. 07070), diluted 1:2 (v:v) into sterile PBS,

and layered on to 1-Step Polymorphs (Accurate Inc. Westbury, NY; catalog no. AN 221,710). The 1-Step Polymorph preparation was centrifuged at 450-500 X g for about 30 minutes at 20°C. The mononuclear cell layer, which was the top layer, was then collected, as was the lower band (which contained neutrophils and eosinophils). All cell samples were then diluted to about 50 ml with PBS, and spun in conical tubes at 780 X g for about 15 minutes.

After spinning, the pellets were placed on ice and about 15 ml of E-Lyse (Cardinal Associates, Santa Fe, NM) was added to each pellet. The pellets were incubated about 10 minutes after which they were washed three times with ice cold PBS and spun at about 200 X g.

The cell pellet was then resuspended in DMEM containing two percent fetal calf serum. Cells were then plated on to 100 mm tissue culture dishes and incubated at 37°C and 10 percent carbon dioxide for 1-3 hours.

After incubation, the unbound cells were removed by washing with PBS. The dendritic cells and monocytes adhered to the plate, while T-cells and B-cells did not. After washing, about 10 ml of DMEM was added to each dish and the dishes were incubated as above. After incubation of about 24 hours, the non-adherent cells, which were mainly dendritic cells, were collected from the plates and counted. The remaining cells adhereing to the plate were primarily monocytes. Any T- or B-cells that contaminated the dendritic cell preparation were removed as follows. Dynabeads (Dynal;Lake Services, NY) conjugated to mouse anti-human CD2 and mouse anti-human CD19 were added in an amount of about 3 beads per cell. Prior to adding the beads to the cells, the beads were washed twice in ice cold PBS, and the cells were resuspended in about 2 ml

of ice cold DMEM containing two percent fetal calf serum. The beads and cells were then combined and rotated at 4°C for 30-60 minutes after which the suspension was placed on a magnet for 5 minutes. The unbound cells, which were the dendritic cells, were then collected, spun, and then cultured in a solution of DMEM containing ten percent fetal calf serum, 50 ng/ml human recombinant GM-CSF (Amgen, Thousand Oaks, CA) and 40 ng/ml human recombinant IL-4 (Amgen, Thousand Oaks, CA). The cells developed the characteristic dendritic appearance in 2-4 days, and were used at days 4-8.

E. Chemoattractant Assay

This assay was used to determine whether TH1 and/or TH2 cells could respond to STCP-1 by migrating towards it in an *in vitro* system.

The assay was conducted using Costar Transwells (5.0 micron porediameter; Costar catalog no. 8421; Cambridge, MA). Human endothelial cell line ECV304 (American Type Culture Collection, catalog no. CRL 1998) were grown to confluence in a medium containing DMEM (Gibco/BRL, Grand Island, NY); 10 percent fetal calf serum (Hyclone Labs, Logan, Utah), penicillin at about 1000 U/ml, streptomycin at about 1000 U/ml (Gibco/BRL), glutamine at about 290 µg/ml, mercaptoethanol at about 50 µM, non-essential amino acids (Gibco/BRL) sodium pyruvate at about 100 µM (Gibco/BRL), and about 10 mM Hepes. At confluence, the cells were plated at a density of about 100,000 cells/well on to Costar Transwell filters (0.5 µm pore size; see above) previously coated with 2 percent gelatin (Sigma, St. Louis, MO). These plates containing the endothelial cells were incubated for two days at about 37°C prior to use to allow the endothelial cells to adhere to the gelatin and to reach confluence.

TH1 and TH2 cells (activated either one, two, or three times) prepared as described above, were centrifuged and resuspended at a density of about 4,000,000 cells/ml in RPMI medium containing 0.5 percent human serum albumin and 10 mM Hepes. About 0.2 ml of this cell suspension was added to the Costar Transwell to which the endothelial cells were adhered. This Transwell filter, the "upper" chamber, was then inserted into the "lower" chamber of the Transwell apparatus. Prior to the insertion of the upper chambers into the lower chambers, 0.4 ml of RPMI medium containing 0.5 percent human serum albumin, 10 mM Hepes, and one chemoattractant (as indicated below), was added to each lower chamber. The chemoattractants used were all human, and, with the exception of STCP-1, were obtained from R and D Systems. The chemoattractants evaluated were:

Eotaxin (100 ng/ml)
Rantes (100 ng/ml)
MIP-1 alpha (100 ng/ml)
MIP-1 beta (100 ng/ml)
STCP-1 (1, 10, or 100 ng/ml as indicated)
STCP-1 polyclonal antibody (20 µg/ml)

The results are shown in Figure 5A-D. TH1 and TH2 cells previously stimulated three times show chemotactic migration towards STCP-1 or RANTES, but not towards eotaxin (Figs. 5B and 5C), while TH2 cells previously stimulated one time are more responsive to RANTES than to STCP-1 (Fig. 5A). TH2 cells exposed to anti-STCP-1 antibody are unable to migrate toward STCP-1 (Fig. 5D).

F. Calcium Flux Assay

To evaluate whether STCP-1 is a chemokine, a standard calcium influx assay for chemokines was conducted as follows.

TH2 cells which had been previously activated twice by culturing them on plates coated with anti-CD3 and anti-CD28 antibodies (see above) were washed in ice cold HBSS (Hanks' balanced salt solution) lacking both divalent cations and phenol red. After washing, the cells were resuspended in the same solution at a concentration of about one million cells per ml and warmed to about 37°C for about 5 minutes. Meanwhile, astandard calcim flux dye, Fluo-3AM (catalog no. F1242; Molecular Probes, Eugene, OR) was reconstituted to about 5 mM in DMSO (dimethyl sulfoxide), and was added to the cell suspension at a concentration of about 5 µM. The cells were incubated in this dye for about 20 minutes at about 37°C. After incubation, the cells were washed three times in ice cold PBS (phosphate buffered saline) containing 1 mM CaCl2, 0.5 mM MgCl2, 10 mM HEPES buffer, and 5.5 mM glucose, and then resuspended in this same buffer at a density of about 1,000,000 cells per ml. The cells were kept at 37°C until use.

Baseline data was obtained on a Becton-Dickinson FACScan as follows. The FACScan was set so that fluorescence 2 intensity was plotted on the Y axis while time was plotted on the X axis. Using FSC vs SSC, the particular cell type of interest was gated on for each experiment. The protocol for running the FACScan was provided by the manufacturer. The acquisition number was set at 150,000 events. For a negative control, about 500 µl of cell suspension was placed in the FACScan, and, after baseline data was obtained, eotaxin at about 100 ng/ml (R and D Systems) was added. For a positive control, the chemokine MCP-1

(R and D Systems) was added at about 100 ng/ml to a separate aliquot of cells after baseline data was obtained on the FACScan for these cells. For the test sample, another aliquot of cells was used; baseline data was obtained, and STCP-1 was then added at about 100 ng/ml. The total time of each run was 102.4 seconds.

The results of FACScan analysis are shown in Figure 6. As can be seen, the TH2 lymphocytes responded to MCP-1 and STCP-1, but not to eotaxin.

Figure 7 shows the results of similar experiments using eosinophils instead of TH2 cells, and adding 100 ng/ml RANTES (R and D Systems, Minneapolis, MN) as an additional positive control. As can be seen, eosinophils responded to eotaxin and RANTES, but not to STCP-1.

Figure 8 shows a histogram plot of the data obtained from FACScans to measure the effect of STCP-1 (100 ng/ml), RANTES (100 ng/ml) C5a (10 mM; Sigma Chemical Co., St. Louis, MO; C5a is a known stimulator of calcium flux in neutrophils) or about 20 µl PBS (-). on calcium flux in neutrophils, dendritic cells, and monocytes as indicated. The data shows the fluorescence shift from the baseline fluorescence obtained after adding the chemokine or C5a. STCP-1 did not appear to have an effect calcium flux in any of the cells tested, while C5a seemed to effect neutrophil calcium flux, and RANTES appeared to effect monocyte and dendritic cell calcium flux.

Deposit of DNA

*E coli* cells containing the plasmid PCRScript SK+ into which the cDNA or genomic DNA encoding full length human STCP-1 has been inserted have been deposited with the ATCC (American Type Culture

Collection, 12301 Parklawn Drive, Rockville, MD, USA) on November 26, 1996 as accession numbers 98268 (cDNA) and 98267 (genomic DNA).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: NOVEL PROTEIN WITH CHEMOKINE ACTIVITY

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 De Havilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 91320

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/760,127
(B) FILING DATE: 03-DEC-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Oleski, Nancy A.
(B) REGISTRATION NUMBER: 34,688
(C) REFERENCE/DOCKET NUMBER: A-429

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (805) 447-6504
(B) TELEFAX: (805) 447-1090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 932 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATACAGGACA GAGCATGGCT CGCCTACAGA CTGCACTCCT GGTTGTCCTC GTCCTCCTTG 60

CTGTGGCGCT TCAAGCAACT GAGGCAGGCC CCTACGGCGC CAACATGGAA GACAGCGTCT 120

GCTGCCGTGA TTACGTCCGT TACCGTCTGC CCCTGCGCGT GGTGAAACAC TTCTACTGGA 180

CCTCAGACTC CTGCCCGAGG CCTGGCGTGG TGTTGCTAAC CTTCAGGGAT AAGGAGATCT 240

GTGCCGATCC CAGAGTGCCC TGGGTGAAGA TGATTCTCAA TAAGCTGAGC CAATGAAGAG 300

CCTACTCTGA TGACCGTGGC CTTGGCTCCT CCAGGAAGGC TCAGGAGCCC TACCTCCCTG 360

CCATTATAGC TGCTCCCCGC CAGAAGCCTG TGCCAACTCT CTGCATTCCC TGATCTCCAT 420

CCCTGTGGCT GTCACCCTTG GTCACCTCCG TGCTGTCACT GCCATCTCCC CCCTGACCCC 480

TCTAACCCAT CCTCTGCCTC CCTCCCTGCA GTCAGAGGGT CCTGTTCCCA TCAGCGATTC 540

CCCTGCTTAA ACCCTTCCAT GACTCCCCAC TGCCCTAAGC TGAGGTCAGT CTCCCAAGCC 600

TGGCATGTGG CCCTCTGGAT CTGGGTTCCA TCTCTGTCTC CAGCCTGCCC ACTTCCCTTC 660

ATGAATGTTG GGTTCTAGCT CCCTGTTCTC CAAACCCATA CTACACATCC CACTTCTGGG 720

TCTTTGCCTG GGATGTTGCT GACACTCAGA AAGTCCCACC ACCTGCACAT GTGTAGCCCC 780

ACCAGCCCTC CAAGGCATTG CTCGCCCAAG CAGCTGGTAA TTCCATTTCA TGTATTAGAT 840

GTCCCCTGGC CCTCTGTCCC CTCTTAATAA CCCTAGTCAC AGTCTCCGCA GATTCTTGGG 900

ATTTGGGGGT TTTCTCCCCC ACCTCTCCAC TA 932

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7146 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1437..1509, 3057..3180, 6120..6201)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGTATTTTTG GTAGAGATGG GGTTTCTCCA TGTTGGCCAG GCTGGTCTTG AACTCCTGAC 60

CTCAGGTGAT CCACCTGCCT GGGCCTTCCA AAGTGCTGGG ATTACAGTCA TGAATCACTG 120

CACCCAGCCC ATGTGTTCTG AGCACCCAAC ATGTGCCTGA TCCTCAGAGG GCCCTGGGAA 180

TGCCAAGAAA AATGAAGGGA GACCCCAGCC TCCTAGAGCT TGGAGTCTGA CTTCTAGGGA 240

GGGGCTTGAG AAAGAGAAAA CCTGACCCAG TCTGCTCAGG GCCACATTCC AGGTCCCCTA 300

GAAGGCTTTG AAGTCCTGAG AGGGTGTGCT TACCAAGGAC ATGACATTTC AAACAGCTTT 360

GACGCATAAG TAGGAGTTTA TGTGGTAGAG ATGTGGAAAG TGACATTAAA GGCCAGGGAC 420

AGGCCGCAGT CCCTTTTGAG GAAGTGTGAG GAGTGCGGTG TGATCCCTCA GGGGAAGGAG 480

ACCAGTGGGG AAAGGCCTGT CCGGAAACGG GGCCTTGAAG GCCACAGACA GGAGCCCGGG 540

ACCTGCCTTT AGGTGAATGG GGAGCCACGG CAGGGTTCTG AAGCAGGAGA GAGAGTGTGT 600

```
AGCTCTGGAC CACTCACGCT GGCTGCTGCA GGGTGGGGAT GGCATTGAGG AGGCCAGCAT      660

CCCAGCCTGA GGTGGAGGTG GCCTGGACTG GGCAGGAACA GTGGGGTTGG AGAGAAGGAG      720

CAGATCTGAG AGAGATTTAG GAAGCAGAAT CAACGGGACA TGGACATGGT GAGGGACTGA      780

GACAGGAGTG GGGGAGGGAG AGCCCGGCAG CCACTGGGAA GGAAGATCAG GCAGGACAGA      840

AGTGTGGGTA GATGCTTTCC ATAAACCGTT GAGAAATCCA CGACCTGGCC CAAATGTGGA      900

CTTCACGAGA AGCCCCAGAT GGGTCAATGG GTCCATAGGC CTCTCCCATT GAAGAGAGAC      960

CAGTAATGCC GTCATTAAGG GTACAAGACA GGCCTCAATT CTAGGTGAGG GTTGGTCCAC     1020

GTTCTCACTT GATTTACAAC CAGTGAAGTC AACAGAGCTG TGAAACCATC TCCCCCATTT     1080

CCAGATGGGG AAACCAAGTC CCAGAGACAC CCAGACATGG CCAAGGCCAC ACAGTGCAGA     1140

GACCCTGGGG AGCACAAGCC TCTAAGAGGG GGATTTTACA AGTCTGTCTG AAACCAGAGG     1200

CGAGGACAGA GTTGAGGGGG GGTCCCCTCT GAGTGGTTCC CCGCCAAAGA GAATTTCTCC     1260

ACTTTGGAGG AAGTGGGAGG TAGTTCTTCT TTTGAGATGT GAATGTCAAG TGACTCTGGG     1320

CACCCCGGTG ACTAAGAGGT AAGTAAGTGA GGCTTGTGGG TGGAGCCAGC ACCTTAAATA     1380

GCAGGTCTTC CTATGTCCCT TTGCAGACAC CTGGGCTGAG ACATACAGGA CAGAGC          1436

ATG GCT CGC CTA CAG ACT GCA CTC CTG GTT GTC CTC GTC CTC CTT GCT        1484
Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala
  1               5                  10                  15

GTG GCG CTT CAA GCA ACT GAG GCA G GTGAGGCTGG GGAGCAGGAA               1529
Val Ala Leu Gln Ala Thr Glu Ala
            20

GACCCCCTAC AGAGGCCAGG GCAGACGGTG GGGTGTCTTC CTCATGTCTT GGACAAGCAC     1589

TGGACCAAGA GCAGAAGACC TCAGTCTGCT GTTGGCTCTT GCGGCCTTCG GCTAGTTGCA     1649

GTGCTTCTCT GGGCCTCAGT TTACAAGTCT GTTGAATGGG CAGTCTTGAA GACTTGGAGT     1709

CTAGGATCTG TCTCACCTGG GTTGGGGGTT GTGGTGGGCA TAGCCTCGGG ATCTCCTGCC     1769

CCAGAAAACT CAAGCATAGG GCCCTAGGGG TGTGGGTGAG GAGCTTCTAA GGGTCAGGTC     1829

TGAGTGGGGG AAGGCAGGGC TGGGGCAAGG GGTTCTCCCT TGGAAAGCTG GTGCTGCCCT     1889

CCCACCATCC ATTTTTTAAA ATGTTATTAT TATTATTTTT TATTTTATTT TATTTTATTT     1949

TTTGAGACAC AGTTTCACTC TTGTTGCCCA GGATGGAGTG CAGTGAGGCA ATCTTGGCCC     2009

ACTGCAACCT CTACCTCCCA GGTTCAAGCG ATTCTCCTGC CTCAGCCTCC TGAGTAGCTG     2069

GGATTACAGG CATGCACCAC CACACCTGGC TAATTTTTGT ATTTTTAGTG GAGACAGGGT     2129

TTCACCATGT TGGCCAGGCT GGTCTCAAAC TCCTGACCTC AGATGATCCT CCCACCTCAG     2189

CCTCCCAAAG TGCTGGGATT ACAGGCATGA GCCACTGCGT CTAGCCTAAA TTTATTTTTT     2249

ATTTCATTTT ATTTATTTAT TTTTTGAGAT GGAGTCTCTG TTGCCCAGGC TGGAGTGCAG     2309
```

```
TGGCGCAATC TCAGCTCACT GCAACCTCTA CCTCTGGAGT TAAAGTGATT CTCCTGCCTC    2369

AGCCTCCTGA GTAGCTGGGA TTACAGGCAC CTGCCACCAT GCCCGGCTAA TTTTTGTATT    2429

TTTAGTAAAG ATGGGGTTTC ACCATGCTGA CCGGTCTTGT CTCGAACTCC TGACCTCAAG    2489

TGAAACTCCC ATCTTGGCCT CCCTAAGTGT TAGGATTACA GGCGTGAGCC ACTGCTCTGG    2549

GCTCACTATC CATTTTTGTC CAGAACCTGT CCTCAGCATG GATTCCTGAC TCCTCTTCTC    2609

CACCCCACTC TATACTTTTG CTGTGTTGGG TGGAGGAGTG AGCACTGGAG TGGGGTCAGG    2669

CAGCTATAAA CAAGAATTGC TCCCAGCTGC TGGGCTTTCT GAGTGCTTTT CATGCATTAA    2729

AGACCCTCTG AAGCTGGTAC TGTTATTACC CCTTTGGTCA GAGGAGGAAA TTGGGCCTCA    2789

GGCTACAGCT GATGCTTAGT TGTTCTGAAT CCCCGCTCCT AGAGCTGACT GTCCCAGAGT    2849

GTAGACATTG GACCCTAAGT GGCTTCTCAG AAGCTCCAAG GGTAGGGTCT TGGAGGGCTC    2909

AGAATCCTCT GGTCACCATC CTTCCACATG AGAACAGAAA AACTGCATCT CTTTCCTGGA    2969

GCCTGGAGAG GCCGAGATCA GGGGCTGGGG CCGAGGGTGA CCTCTCTGGG CTCCAGCTTG    3029

TGAATTCACT GGGGACCCCT CCCCTAG  GC CCC TAC GGC GCC AAC ATG GAA         3079
                                 Gly Pro Tyr Gly Ala Asn Met Glu
                                  25                  30

GAC AGC GTC TGC TGC CGT GAT TAC GTC CGT TAC CGT CTG CCC CTG CGC      3127
Asp Ser Val Cys Cys Arg Asp Tyr Val Arg Tyr Arg Leu Pro Leu Arg
         35                  40                  45

GTG GTG AAA CAC TTC TAC TGG ACC TCA GAC TCC TGC CCG AGG CCT GGC      3175
Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly
     50                  55                  60

GTG GT  GTGAGTAGGG AGCTGGGGCC ACAGGGCCTT GGTGGGCCTG ACGGGTACAG      3230
Val Val
 65

CCTGGGATGG CCCAGGTGCT GGTGGGTGGG ACACACCCAG GGATGAGAGG AATGTGGCAG    3290

GGCTACCAGA TGCCTGCCAG GATGGCTTGG CTGGAAGAGA TGGCTCAGTT CAGGCTTGGG    3350

TGGACTACAA ACAAAAATAA TGTGATCGTT TAGCCAATAT CCTCAGGCGC TTACTAGGTG    3410

CCAGGCAACA TGTCAGGTTC TGAGGATGCA GAGCTGAGTG AACAGGGTGC CACCAGAGTG    3470

TGGGTATGGC AGTAGGTAGT CAGTAGCTGT GGCATCTAGG GTATTTGGGT ATAGCAGGTA    3530

TAGGAGTATA CCTAGTGCCA CTGAGTCAGC AAAGATGCTT CCAGGTTCTG GGCAAAAATG    3590

GATGGTGAAC CAGGACAGGC GTGGTGGCTC ATGCCTGTAA TCCCAGCATT TTGGGAGGCC    3650

GAGGCAGGCA GATCACTTGA GGTCAGGAGT TCAAGATCAG CCTGGTCAAC ATGGTGAAAC    3710

CCCATCTCTA CTAAAAATAC AAAATTAGCT GAGTGTGATG GTGCGTGCCT GTGGTCCCAG    3770

CTACTTGGGA GGCTGAGGCA GGAGAATCAT TTGAACCTGG AAGGTGGAGG TTGCAGTGAG    3830

CCGAGATCGC TCCACCTCAC TCCAGCCTGG GCAACAGAGG GAGACTCTGT CTCAAAAAAA    3890

AAAAAAAAAA ATGCTCACAC CAGAAGTGTC TTGGATCCTC TCCAGGCTCC CAGAAGTCCA    3950
```

AGGCAGCCCA TTTTGACCAG AAGAATAACA TTAAGAGCTA AAATTTCCAA GGTGCCTCCC 4010

GAGTGCCAGG CACTGTTCTG ATCATTACCT GTGTTAACTG GTTTAATTCT CACAACAATC 4070

CTACGAGGAA GTTCAACGAT TCCCATGGTA GAGATGGAGA AACAGGCTTA GCAAGGGACA 4130

GTGACCTGCT CAAGGCTGCC CAGGTTGGAG CCAGAACTCA CTCCTGGTTC CTCGTTCAGG 4190

GCTTTCCCCT GAGTTTCTTG TTCTCCTTTG CTTCTTTCTC TGGCTTCTTT GCCTCCTTTT 4250

CCCTGGCCTG GGGCCTGCAC CTGCACCTGG CTGGGTGACA AGTCCTGCCC TCTCTGCGGT 4310

AGCCTCTCTG GCTGCTTCTC CAACTGCTCA GAGCCTGCTG CCTACCAAAT CTCACACCTG 4370

GGAAGGCTGG GTTTGGGGAC TCATGACCCA CTTTGGGCCT CTATTATCTT CTCATCTTCC 4430

TCCTCCTTAT TGCTGACACC ATCTCTTAGA GGGATCTGCA GGTGAATAAT AAAAAAGGCT 4490

GAAGCAGGAA GCCCTCCCAG AGTTCTTGTC TCTTTAACTC TGAGCCTCAG TTTCCCCAAC 4550

AGTATAATGA AGTAATAACC TAAACTTATT TGACTTATTT GTATTTATCA AACACATAGA 4610

GAGTGCTTGC TAAGTGCTAG GCTCTGCCGT AAGCACTTTA TAAATATGAA CTCATTTAAT 4670

CCTTGAAACA ATCCTATGCA GTAGGTGCCA TCGTGACCCC CTTTTCACAG GTGAGGAAAT 4730

GAGCACAAAA AGGTTAGGGG GCCTCTTGAG CATTACAGGG CACAGTAATA GTAAGAGGAA 4790

GGTGAAGAGC TCAATGTCTG GCACATAATA GATGCTCAAT TGCTGGGCAT GTAGTGCTTC 4850

TCAGTTACTT GGGAGGCTGA GGCAGGAGGA TTTCCTGAGC CCTGGATTTT GAGGCCAGCC 4910

TGGAAACTTA CTGAGACTCT GTCTTTATTT CATTTTTTTA AATAAAAAAG GCTGAGTGCA 4970

GTGGCTTACA CCTGTAATCT CAGGACTTTG GGAGGCTGAG TTGGGAGGAT CACTTGAGGC 5030

CAGGAGTTCA AGACAAGCCT CAATAGAGTG AGACCCTGTC TCTAAAATCA ATCAATCAAT 5090

CAATCAAATC ATATGTGATC ACTGGATTTT GAGCCAGAAG GGACCCTAGA AAGAATGTGT 5150

CTGGAGGCTC AGAGTAGTTA AGTGATATTA TAAGACTCTT GGGGATTCTG GCTTGGCGCA 5210

GTGGCTCACT CCTATAATCC TAGCCTTTGG GAGGCCGAGA CAGGCAGGAT CACTTGAGGT 5270

CAGAAGTTCA AGAGCAGCCT GGCCAACATG GTGAAAACCT ATCTCTACTA AAAATACAAA 5330

AATTAGCTGG GTGTTGTGGC GGGCACCTGT AATCCCGGCT ACTCGGGAGC CTGAGGCAGG 5390

AGGATCGCCT GAACCCGGGA GTTGGAGGTT GCAGTGAGCC AAGATCGTAC CACTACACTC 5450

CAGCCTGGGT GACAGAGACT CCATCTCAAA AAATAAAAAC AAAACAAAAC AAAAACAAAA 5510

ACAAAACCCA ACTCTTGGGG ATTCTATTTC ATGGCCTGAT CTGGAACTCA AGGCTGGGAT 5570

TCAAGAGCTG AGGGTCCTGG AAGTCCTTGC TGCCTCTTCC TCTTTTTTTT TTTCTTGACA 5630

CGGGGTCTCA TTCTGTTGCC TAGGCTGGAG TACAGTGGAG CAGAGATCTG GGCTCACTGC 5690

AGCCTTGACC TCCTGAGTCC AAGCAATCCT CCCACCTCAG CCTCCCAAGT AGCTGAGATC 5750

ACAGGCAGAT GTCACCATGC CTGGCTGACT TGTACGTTTT TGTAGAGACA GGGTTTTGCC 5810

```
ATGTTGCCCA GGATGGCCTT AAACCCCTAG GCTCAAACAA TCCTCCTGCC TCGGCCTCCC    5870

AAAGTGCTGG GATTACAGGC GTGAACTCCT GGCCTCCTCT TCCTCCTGAG AAATATTCTT    5930

TTCACACCAC AGGTGGCTTG TAAATTTTGA ACCACTCTAT TTAGCAGATA ATGTCATATG    5990

TAGTAGGTGA CTCATAAATG CTGAGCCCTG CATAAAGTAG GTGGCTCATA AATGCTAAGC    6050

TCCCGAGGGT GTGGCATCCT TGCTGCGTGC TAATGTTGCT GCATTGTCTC TGGGGTCTCC    6110

TTCTTCCAG G TTG CTA ACC TTC AGG GAT AAG GAG ATC TGT GCC GAT CCC       6159
            Leu Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro
                        70                  75

AGA GTG CCC TGG GTG AAG ATG ATT CTC AAT AAG CTG AGC CAA              6201
Arg Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu Ser Gln
 80                  85                  90

TGAAGAGCCT ACTCTGATGA CCGTGGCCTT GGCTCCTCCA GGAAGGCTCA GGAGCCCTAC    6261

CTCCCTGCCA TTATAGCTGC TCCCCGCCAG AAGCCTGTGC CAACTCTCTG CATTCCCTGA    6321

TCTCCATCCC TGTGGCTGTC ACCCTTGGTC ACCTCCGTGC TGTCACTGCC ATCTCCCCCC    6381

TGACCCCTCT AACCCATCCT CTGCCTCCCT CCCTGCAGTC AGAGGGTCCT GTTCCCATCA    6441

GCGATTCCCC TGCTTAAACC CTTCCATGAC TCCCCACTGC CCTAAGCTGA GGTCAGTCTC    6501

CCAAGCCTGG CATGTGGCCC TCTGGATCTG GGTTCCATCT CTGTCTCCAG CCTGCCCACT    6561

TCCCTTCATG AATGTTGGGT TCTAGCTCCC TGTTCTCCAA ACCCATACTA CACATCCCAC    6621

TTCTGGGTCT TTGCCTGGGA TGTTGCTGAC ACTCAGAAAG TCCCACCACC TGCACATGTG    6681

TAGCCCCACC AGCCCTCCAA GGCATTGCTC GCCCAAGCAG CTGGTAATTC CATTTCATGT    6741

ATTAGATGTC CCCTGGCCCT CTGTCCCCTC TTAATAACCC TAGTCACAGT CTCCGCAGAT    6801

TCTTGGGATT TGGGGGTTTT CTCCCCCACC TCTCCACTAG TTGGACCAAG GTTTCTAGCT    6861

AAGTTACTCT AGTCTCCAAG CCTCTAGCAT AGAGCACTGC AGACAGGCCC TGGCTCAGAA    6921

TCAGAGCCCA GAAAGTGGCT GCAGACAAAA TCAATAAAAC TAATGTCCCT CCCCTCTCCC    6981

TGCCAAAAGG CAGTTACATA TCAATACAGA GACTCAAGGT CACTAGAAAT GGGCCAGCTG    7041

GGTCAATGTG AAGCCCCAAA TTTGCCCAGA TTCACCTTTC TTCCCCCACT CCCTTTTTTT    7101

TTTTTTTTTT GAGATGGAGT TTCGCTCTTG TCACCCACGC TGGAG                    7146
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala
 1               5                  10                  15

Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr Gly Ala Asn Met Glu
            20                  25                  30

Asp Ser Val Cys Cys Arg Asp Tyr Val Arg Tyr Arg Leu Pro Leu Arg
        35                  40                  45

Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly
    50                  55                  60

Val Val Leu Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro Arg
65                  70                  75                  80

Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu Ser Gln
                85                  90
```

WE CLAIM:

1. A nucleic acid molecule encoding a polypeptide selected from the group consisting of:

(a) the nucleic acid molecule of SEQ ID NO:1;

(b) the nucleic acid molecule of SEQ ID NO:2;

(c) a nucleic acid molecule encoding the polypeptide of SEQ ID NO:3 or a biologically active fragment thereof;

(d) a nucleic acid molecule that encodes a polypeptide that is at least 70 percent identical to the polypeptide of SEQ ID NO:3;

(e) a nucleic acid molecule that hybridizes under stringent conditions to any of (a)-(d) above; and

(f) a nucleic acid molecule that is the complement of any of (a)-(e) above.

2. The nucleic acid molecule that is SEQ ID NO:1.

3. The nucleic acid molecule that is SEQ ID NO:2.

4. A nucleic acid molecule encoding the polypeptide of SEQ ID NO:3.

5. A nucleic acid molecule encoding amino acids 25-93 of SEQ ID NO:3

6. A vector comprising the nucleic acid molecule of claim 1.

7. A vector comprising the nucleic acid molecule of claim 2.

8. A vector comprising the nucleic acid molecule of claim 3.

9. A vector comprising the nucleic acid molecule of claim 4.

10. A vector comprising the nucleic acid molecule of claim 5.

11. A host cell comprising the vector of claim 6.

12. A host cell comprising the vector of claim 7.

13. A host cell comprising the vector of claim 8.

14. A host cell comprising the vector of claim 9.

15. A host cell comprising the vector of claim 10.

16. A process for producing a STCP-1 polypeptide comprising the steps of:

(a) expressing a polypeptide encoded by the nucleic acid of claim 1 in a suitable host; and

(b) isolating the polypeptide.

17. The process of claim 13 wherein the polypeptide is SEQ ID NO:3.

18. The process of claim 13 wherein the polypeptide is amino acids 25-93 of SEQ ID NO:3.

19. A STCP-1 polypeptide selected from the group consisting of:

(a) the polypeptide of SEQ ID NO:3;

(b) the polypeptide that is amino acids 25-93 of SEQ ID NO:3; and

(c) a polypeptide that is at least 70 percent identical to the polypeptide of (a) or (b).

20. A STCP-1 polypeptide that is the polypeptide of SEQ ID NO:3 or a biologically active fragment thereof.

21. The STCP-1 polypeptide of claim 20 that does not possess an amino terminal methionine.

22. An antibody or fragment thereof which specifically binds human STCP-1.

23. The antibody of claim 22 that is a monoclonal antibody.

# NOVEL PROTEIN WITH CHEMOKINE ACTIVITY

## ABSTRACT OF THE DISCLOSURE

Disclosed are nucleic acids encoding a novel chemokine that is specific for T-cells. Also disclosed are the amino acid sequence of the chemokine, and methods for preparing the chemokine.

FIG.1A

```
   1 ATACAGGACA GAGCATGGCT CGCCTACAGA CTGCACTCCT GGTTGTCCTC
  51 GTCCTCCTTG CTGTGGCGCT TCAAGCAACT GAGGCAGGCC CCTACGGCGC
 101 CAACATGGAA GACAGCGTCT GCTGCCGTGA TTACGTCCGT TACCGTCTGC
 151 CCCTGCGCGT GGTGAAACAC TTCTACTGGA CCTCAGACTC CTGCCCGAGG
 201 CCTGGCGTGG TGTTGCTAAC CTTCAGGGAT AAGGAGATCT GTGCCGATCC
 251 CAGAGTGCCC TGGGTGAAGA TGATTCTCAA TAAGCTGAGC CAATGAAGAG
 301 CCTACTCTGA TGACCGTGGC CTTGGCTCCT CCAGGAAGGC TCAGGAGCCC
 351 TACCTCCCTG CCATTATAGC TGCTCCCCGC CAGAAGCCTG TGCCAACTCT
 401 CTGCATTCCC TGATCTCCAT CCCTGTGGCT GTCACCCTTG GTCACCTCCG
 451 TGCTGTCACT GCCATCTCCC CCCTGACCCC TCTAACCCAT CCTCTGCCTC
```

FIG.1B

```
501  CCTCCCTGCA GTCAGAGGGT CCTGTTCCCA TCAGCGATTC CCCTGCTTAA
551  ACCCTTCCAT GACTCCCCAC TGCCCTAAGC TGAGGTCAGT CTCCCAAGCC
601  TGGCATGTGG CCCTCTGGAT CTGGGTTCCA TCTCTGTCTC CAGCCTGCCC
651  ACTTCCCTTC ATGAATGTTG GGTTCTAGCT CCCTGTTCTC CAAACCCATA
701  CTACACATCC CACTTCTGGG TCTTTGCCTG GGATGTTGCT GACACTCAGA
751  AAGTCCCACC ACCTGCACAT GTGTAGCCCC ACCAGCCCTC CAAGGCATTG
801  CTCGCCCAAG CAGCTGGTAA TTCCATTTCA TGTATTAGAT GTCCCCTGGC
851  CCTCTGTCCC CTCTTAATAA CCCTAGTCAC AGTCTCCGCA GATTCTTGGG
901  ATTTGGGGGT TTTCTCCCCC ACCTCTCCAC TA
```

FIG.2A

```
1    TGTATTTTTG GTAGAGATGG GGTTTCTCCA TGTTGGCCAG GCTGGTCTTG
51   AACTCCTGAC CTCAGGTGAT CCACCTGCCT GGGCCTTCCA AAGTGCTGGG
101  ATTACAGTCA TGAATCACTG CACCCAGCCC ATGTGTTCTG AGCACCCAAC
151  ATGTGCCTGA TCCTCAGAGG GCCCTGGGAA TGCCAAGAAA AATGAAGGGA
201  GACCCCAGCC TCCTAGAGCT TGGAGTCTGA CTTCTAGGGA GGGGCTTGAG
251  AAAGAGAAAA CCTGACCCAG TCTGCTCAGG GCCACATTCC AGGTCCCCTA
301  GAAGGCTTTG AAGTCCTGAG AGGGTGTGCT TACCAAGGAC ATGACATTTC
351  AAACAGCTTT GACGCATAAG TAGGAGTTTA TGTGGTAGAG ATGTGGAAAG
401  TGACATTAAA GGCCAGGGAC AGGCCGCAGT CCCTTTTGAG GAAGTGTGAG
451  GAGTGCGGTG TGATCCCTCA GGGGAAGGAG ACCAGTGGGG AAAGGCCTGT
501  CCGGAAACGG GGCCTTGAAG GCCACAGACA GGAGCCCGGG ACCTGCCTTT
551  AGGTGAATGG GGAGCCACGG CAGGGTTCTG AAGCAGGAGA GAGAGTGTGT
```

FIG.2A -1

```
 601  AGCTCTGGAC CACTCACGCT GGCTGCTGCA GGGTGGGGAT GGCATTGAGG
 651  AGGCCAGCAT CCCAGCCTGA GGTGGAGGTG GCCTGGACTG GGCAGGAACA
 701  GTGGGGTTGG AGAGAAGGAG CAGATCTGAG AGAGATTTAG GAAGCAGAAT
 751  CAACGGGACA TGGACATGGT GAGGGACTGA GACAGGAGTG GGGGAGGGAG
 801  AGCCCGGCAG CCACTGGGAA GGAAGATCAG GCAGGACAGA AGTGTGGGTA
 851  GATGCTTTCC ATAAACCGTT GAGAAATCCA CGACCTGGCC CAAATGTGGA
 901  CTTCACGAGA AGCCCCAGAT GGGTCAATGG GTCCATAGGC CTCTCCCATT
 951  GAAGAGAGAC CAGTAATGCC GTCATTAAGG GTACAAGACA GGCCTCAATT
1001  CTAGGTGAGG GTTGGTCCAC GTTCTCACTT GATTTACAAC CAGTGAAGTC
1051  AACAGAGCTG TGAAACCATC TCCCCCATTT CCAGATGGGG AAACCAAGTC
1101  CCAGAGACAC CCAGACATGG CCAAGGCCAC ACAGTGCAGA GACCCTGGGG
1151  AGCACAAGCC TCTAAGAGGG GGATTTTACA AGTCTGTCTG AAACCAGAGG
```

FIG.2B

```
1201  CGAGGACAGA GTTGAGGGGG GGTCCCCCTCT GAGTGGTTCC CCGCCAAAGA
1251  GAATTTCTCC ACTTTGGAGG AAGTGGGAGG TAGTTCTTCT TTTGAGATGT
1301  GAATGTCAAG TGACTCTGGG CACCCCGGTG ACTAAGAGGT AAGTAAGTGA
1351  GGCTTGTGGG TGGAGCCAGC ACCTTAAATA GCAGGTCTTC CTATGTCCCT
1401  TTGCAGACAC CTGGGCTGAG ACATACAGGA CAGAGCATGG CTCGCCTACA
1451  GACTGCACTC CTGGTTGTCC TCGTCCTCCT TGCTGTGGCG CTTCAAGCAA
1501  CTGAGGCAGG TGAGGCTGGG GAGCAGGAAG ACCCCCTACA GAGGCCAGGG
1551  CAGACGGTGG GGTGTCTTCC TCATGTCTTG GACAAGCACT GGACCAAGAG
1601  CAGAAGACCT CAGTCTGCTG TTGGCTCTTG CGGCCTTCGG CTAGTTGCAG
1651  TGCTTCTCTG GGCCTCAGTT TACAAGTCTG TTGAATGGGC AGTCTTGAAG
1701  ACTTGGAGTC TAGGATCTGT CTCACCTGGG TTGGGGGTTG TGGTGGGCAT
1751  AGCCTCGGGA TCTCCTGCCC CAGAAAACTC AAGCATAGGG CCCTAGGGGT
```

FIG.2B -1

```
1801  GTGGGTGAGG AGCTTCTAAG GGTCAGGTCT GAGTGGGGGA AGGCAGGGCT
1851  GGGGCAAGGG GTTCTCCCTT GGAAAGCTGG TGCTGCCCTC CCACCATCCA
1901  TTTTTTAAAA TGTTATTATT ATTATTTTTT ATTTTATTTT ATTTTATTTT
1951  TTGAGACACA GTTTCACTCT TGTTGCCCAG GATGGAGTGC AGTGAGGCAA
2001  TCTTGGCCCA CTGCAACCTC TACCTCCCAG GTTCAAGCGA TTCTCCTGCC
2051  TCAGCCTCCT GAGTAGCTGG GATTACAGGC ATGCACCACC ACACCTGGCT
2101  AATTTTTGTA TTTTTAGTGG AGACAGGGTT TCACCATGTT GGCCAGGCTG
2151  GTCTCAAACT CCTGACCTCA GATGATCCTC CCACCTCAGC CTCCCAAAGT
2201  GCTGGGATTA CAGGCATGAG CCACTGCGTC TAGCCTAAAT TTATTTTTTA
2251  TTTCATTTTA TTTATTTATT TTTTGAGATG GAGTCTCTGT TGCCCAGGCT
2301  GGAGTGCAGT GGCGCAATCT CAGCTCACTG CAACCTCTAC CTCTGGAGTT
2351  AAAGTGATTC TCCTGCCTCA GCCTCCTGAG TAGCTGGGAT TACAGGCACC
```

FIG.2C

```
2401  TGCCACCATG CCCGGCTAAT TTTTGTATTT TTAGTAAAGA TGGGGTTTCA
2451  CCATGCTGAC CGGTCTTGTC TCGAACTCCT GACCTCAAGT GAAACTCCCA
2501  TCTTGGCCTC CCTAAGTGTT AGGATTACAG GCGTGAGCCA CTGCTCTGGG
2551  CTCACTATCC ATTTTTGTCC AGAACCTGTC CTCAGCATGG ATTCCTGACT
2601  CCTCTTCTCC ACCCCACTCT ATACTTTTGC TGTGTTGGGT GGAGGAGTGA
2651  GCACTGGAGT GGGGTCAGGC AGCTATAAAC AAGAATTGCT CCCAGCTGCT
2701  GGGCTTTCTG AGTGCTTTTC ATGCATTAAA GACCCTCTGA AGCTGGTACT
2751  GTTATTACCC CTTTGGTCAG AGGAGGAAAT TGGGCCTCAG GCTACAGCTG
2801  ATGCTTAGTT GTTCTGAATC CCCGCTCCTA GAGCTGACTG TCCCAGAGTG
2851  TAGACATTGG ACCCTAAGTG GCTTCTCAGA AGCTCCAAGG GTAGGGTCTT
2901  GG[illegible]GCTCA GAATCCTCTG GTCACCATCC TTCCACATGA GAACAGAAAA
2951  ACTGCATCTC T[illegible]TGGAG CCTGGAGAGG CCGAGATCAG GGGCTGGGGC
```

FIG. 2C - 1

```
3001  CGAGGGTGAC CTCTCTGGGC TCCAGCTTGT GAATTCACTG GGGACCCCTC
3051  CCCTAGGCCC CTACGGCGCC AACATGGAAG ACAGCGTCTG CTGCCGTGAT
3101  TACGTCCGTT ACCGTCTGCC CCTGCGCGTG GTGAAACACT TCTACTGGAC
3151  CTCAGACTCC TGCCCGAGGC CTGGCGTGGT GTGAGTAGGG AGCTGGGGCC
3201  ACAGGGCCTT GGTGGGCCTG ACGGGTACAG CCTGGGATGG CCCAGGTGCT
3251  GGTGGGTGGG ACACACCCAG GGATGAGAGG AATGTGGCAG GGCTACCAGA
3301  TGCCTGCCAG GATGGCTTGG CTGGAAGAGA TGGCTCAGTT CAGGCTTGGG
3351  TGGACTACAA ACAAAAATAA TGTGATCGTT TAGCCAATAT CCTCAGGCGC
3401  TTACTAGGTG CCAGGCAACA TGTCAGGTTC TGAGGATGCA GAGCTGAGTG
3451  AACAGGGTGC CACCAGAGTG TGGGTATGGC AGTAGGTAGT CAGTAGCTGT
3501  GGCATCTAGG GTATTTGGGT ATAGCAGGTA TAGGAGTATA CCTAGTGCCA
3551  CTGAGTCAGC AAAGATGCTT CCAGGTTCTG GGCAAAAATG GATGGTGAAC
```

FIG.2D

```
3601  CAGGACAGGC GTGGTGGCTC ATGCCTGTAA TCCCAGCATT TTGGGAGGCC
3651  GAGGCAGGCA GATCACTTGA GGTCAGGAGT TCAAGATCAG CCTGGTCAAC
3701  ATGGTGAAAC CCCATCTCTA CTAAAAATAC AAAATTAGCT GAGTGTGATG
3751  GTGCGTGCCT GTGGTCCCAG CTACTTGGGA GGCTGAGGCA GGAGAATCAT
3801  TTGAACCTGG AAGGTGGAGG TTGCAGTGAG CCGAGATCGC TCCACCTCAC
3851  TCCAGCCTGG GCAACAGAGG GAGACTCTGT CTCAAAAAAA AAAAAAAAAA
3901  ATGCTCACAC CAGAAGTGTC TTGGATCCTC TCCAGGCTCC CAGAAGTCCA
3951  AGGCAGCCCA TTTTGACCAG AAGAATAACA TTAAGAGCTA AAATTTCCAA
4001  GGTGCCTCCC GAGTGCCAGG CACTGTTCTG ATCATTACCT GTGTTAACTG
4051  GTTTAATTCT CACAACAATC CTACGAGGAA GTTCAACGAT TCCCATGGTA
4101  GAGATGGAGA AACAGGCTTA GCAAGGGACA GTGACCTGCT CAAGGCTGCC
4151  CAGGTTGGAG CCAGAACTCA CTCCTGGTTC CTCGTTCAGG GCTTTCCCCT
```

FIG.2D-1

```
4201  GAGTTTCTTG TTCTCCTTTG CTTCTTTCTC TGGCTTCTTT GCCTCCTTTT
4251  CCCTGGCCTG GGGCCTGCAC CTGCACCTGG CTGGGTGACA AGTCCTGCCC
4301  TCTCTGCGGT AGCCTCTCTG GCTGCTTCTC CAACTGCTCA GAGCCTGCTG
4351  CCTACCAAAT CTCACACCTG GGAAGGCTGG GTTTGGGGAC TCATGACCCA
4401  CTTTGGGCCT CTATTATCTT CTCATCTTCC TCCTCCTTAT TGCTGACACC
4451  ATCTCTTAGA GGGATCTGCA GGTGAATAAT AAAAAAGGCT GAAGCAGGAA
4501  GCCCTCCCAG AGTTCTTGTC TCTTTAACTC TGAGCCTCAG TTTCCCCAAC
4551  AGTATAATGA AGTAATAACC TAAACTTATT TGACTTATTT GTATTTATCA
4601  AACACATAGA GAGTGCTTGC TAAGTGCTAG GCTCTGCCGT AAGCACTTTA
4651  TAAATATGAA CTCATTTAAT CCTTGAAACA ATCCTATGCA GTAGGTGCCA
4701  TCGTGACCCC CTTTTCACAG GTGAGGAAAT GAGCACAAAA AGGTTAGGGG
4751  GCCTCTTGAG CATTACAGGG CACAGTAATA GTAAGAGGAA GGTGAAGAGC
```

FIG.2E

```
4801  TCAATGTCTG GCACATAATA GATGCTCAAT TGCTGGGCAT GTAGTGCTTC
4851  TCAGTTACTT GGGAGGCTGA GGCAGGAGGA TTTCCTGAGC CCTGGATTTT
4901  GAGGCCAGCC TGGAAACTTA CTGAGACTCT GTCTTTATTT CATTTTTTTA
4951  AATAAAAAAG GCTGAGTGCA GTGGCTTACA CCTGTAATCT CAGGACTTTG
5001  GGAGGCTGAG TTGGGAGGAT CACTTGAGGC CAGGAGTTCA AGACAAGCCT
5051  CAATAGAGTG AGACCCTGTC TCTAAAAATCA ATCAATCAAT CAATCAAATC
5101  ATATGTGATC ACTGGATTTT GAGCCAGAAG GGACCCTAGA AAGAATGTGT
5151  CTGGAGGCTC AGAGTAGTTA AGTGATATTA TAAGACTCTT GGGGATTCTG
5201  GCTTGGCGCA GTGGCTCACT CCTATAATCC TAGCCTTTGG GAGGCCGAGA
5251  CAGGCAGGAT CACTTGAGGT CAGAAGTTCA AGAGCAGCCT GGCCAACATG
5301  GTGAAAACCT ATCTCTACTA AAAATACAAA AATTAGCTGG GTGTTGTGGC
5351  GGGCACCTGT AATCCCGGCT ACTCGGGAGC CTGAGGCAGG AGGATCGCCT
```

FIG.2E -1

```
5401  GAACCCGGGA GTTGGAGGTT GCAGTGAGCC AAGATCGTAC CACTACACTC
5451  CAGCCTGGGT GACAGAGACT CCATCTCAAA AAATAAAAAC AAAACAAAAC
5501  AAAAACAAAA ACAAAACCCA ACTCTTGGGG ATTCTATTTC ATGGCCTGAT
5551  CTGGAACTCA AGGCTGGGAT TCAAGAGCTG AGGGTCCTGG AAGTCCTTGC
5601  TGCCTCTTCC TCTTTTTTTT TTTCTTGACA CGGGGTCTCA TTCTGTTGCC
5651  TAGGCTGGAG TACAGTGGAG CAGAGATCTC GGCTCACTGC AGCCTTGACC
5701  TCCTGAGTCC AAGCAATCCT CCCACCTCAG CCTCCCAAGT AGCTGAGATC
5751  ACAGGCAGAT GTCACCATGC CTGGCTGACT TGTACGTTTT TGTAGAGACA
5801  GGGTTTTGCC ATGTTGCCCA GGATGGCCTT AAACCCCTAG GCTCAAACAA
5851  TCCTCCTGCC TCGGCCTCCC AAAGTGCTGG GATTACAGGC GTGAACTCCT
5901  GGCCTCCTCT TCCTCCTGAG AAATATTCTT TTCACACCAC AGGTGGCTTG
5951  TAAATTTTGA ACCACTCTAT TTAGCAGATA ATGTCATATG TAGTAGGTGA
```

FIG. 2F

```
6001  CTCATAAATG CTGAGCCCTG CATAAAGTAG GTGGCTCATA AATGCTAAGC
6051  TCCCGAGGGT GTGGCATCCT TGCTGCGTGC TAATGTTGCT GCATTGTCTC
6101  TGGGGTCTCC TTCTTCCAGG TTGCTAACCT TCAGGGATAA GGAGATCTGT
6151  GCCGATCCCA GAGTGCCCTG GGTGAAGATG ATTCTCAATA AGCTGAGCCA
6201  ATGAAGAGCC TACTCTGATG ACCGTGGCCT TGGCTCCTCC AGGAAGGCTC
6251  AGGAGCCCTA CCTCCCTGCC ATTATAGCTG CTCCCCGCCA GAAGCCTGTG
6301  CCAACTCTCT GCATTCCCTG ATCTCCATCC CTGTGGCTGT CACCCTTGGT
6351  CACCTCCGTG CTGTCACTGC CATCTCCCCC CTGACCCCTC TAACCCATCC
6401  TCTGCCTCCC TCCCTGCAGT CAGAGGGTCC TGTTCCCATC AGCGATTCCC
6451  CTGCTTAAAC CCTTCCATGA CTCCCCCACTG CCCTAAGCTG AGGTCAGTCT
6501  CCCAAGCCTG GCATGTGGCC CTCTGGATCT GGGTTCCATC TCTGTCTCCA
6551  GCCTGCCCAC TTCCCTTCAT GAATGTTGGG TTCTAGCTCC CTGTTCTCCA
```

FIG.2F -1

```
6601  AACCCATACT ACACATCCCA CTTCTGGGTC TTTGCCTGGG ATGTTGCTGA
6651  CACTCAGAAA GTCCCACCAC CTGCACATGT GTAGCCCCAC CAGCCCTCCA
6701  AGGCATTGCT CGCCCAAGCA GCTGGTAATT CCATTTCATG TATTAGATGT
6751  CCCCTGGCCC TCTGTCCCCT CTTAATAACC CTAGTCACAG TCTCCGCAGA
6801  TTCTTGGGAT TTGGGGGTTT TCTCCCCCAC CTCTCCACTA GTTGGACCAA
6851  GGTTTCTAGC TAAGTTACTC TAGTCTCCAA GCCTCTAGCA TAGAGCACTG
6901  CAGACAGGCC CTGGCTCAGA ATCAGAGCCC AGAAAGTGGC TGCAGACAAA
6951  ATCAATAAAA CTAATGTCCC TCCCCTCTCC CTGCCAAAAG GCAGTTACAT
7001  ATCAATACAG AGACTCAAGG TCACTAGAAA TGGGCCAGCT GGGTCAATGT
7051  GAAGCCCCAA ATTTGCCCAG ATTCACCTTT CTTCCCCCAC TCCCTTTTTT
7101  TTTTTTTTTT TGAGATGGAG TTTCGCTCTT GTCACCCACG CTGGAG
```

FIG.3

```
1   MARLQTALLV VLVLLAVALQ ATEAGPYGAN MEDSVCCRDY VRYRLPLRVV

51  KHFYWTSDSC PRPGVVLLTF RDKEICADPR VPWVKMILNK LSQ*
```

FIG. 4

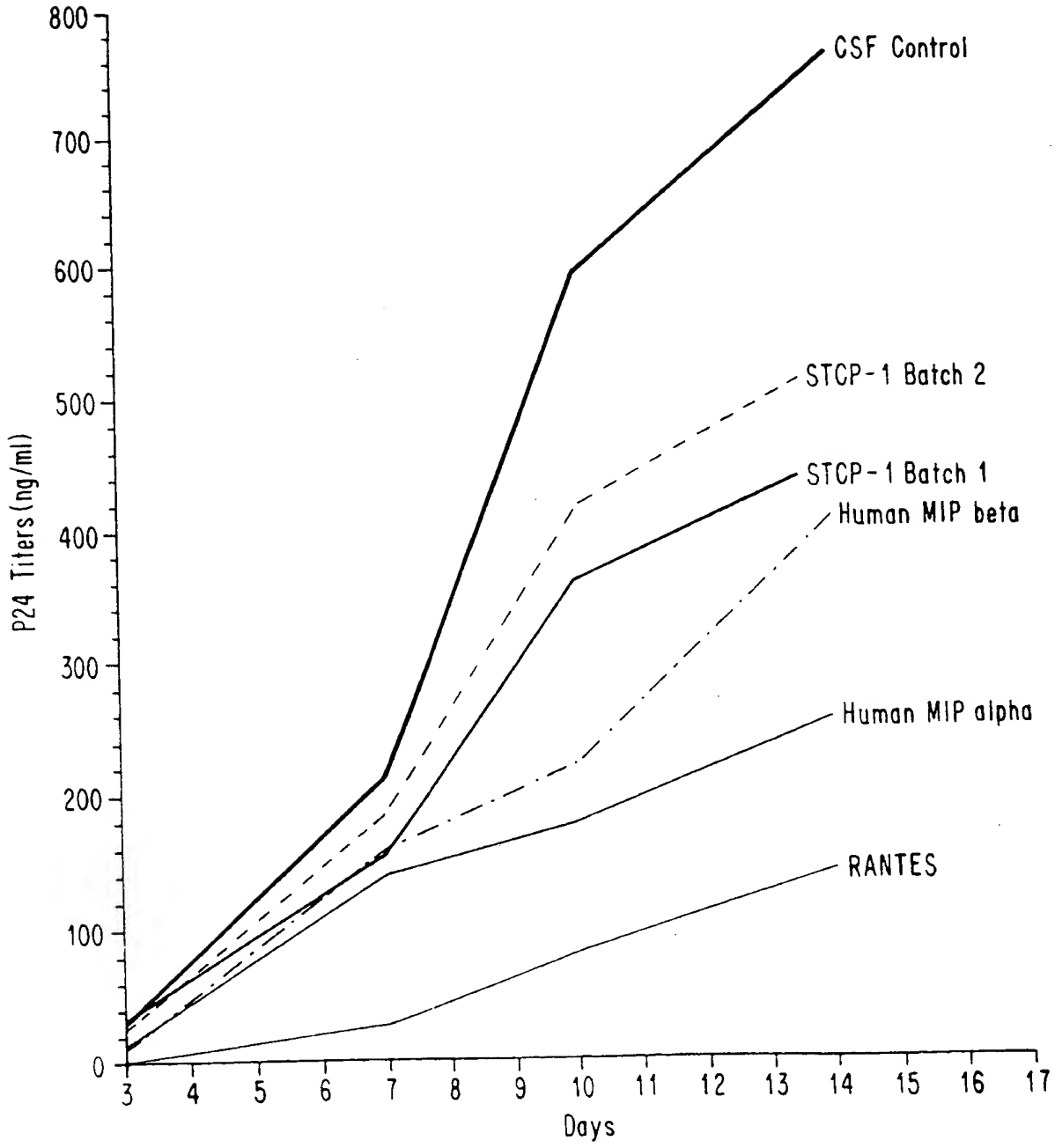


FIG. 5A

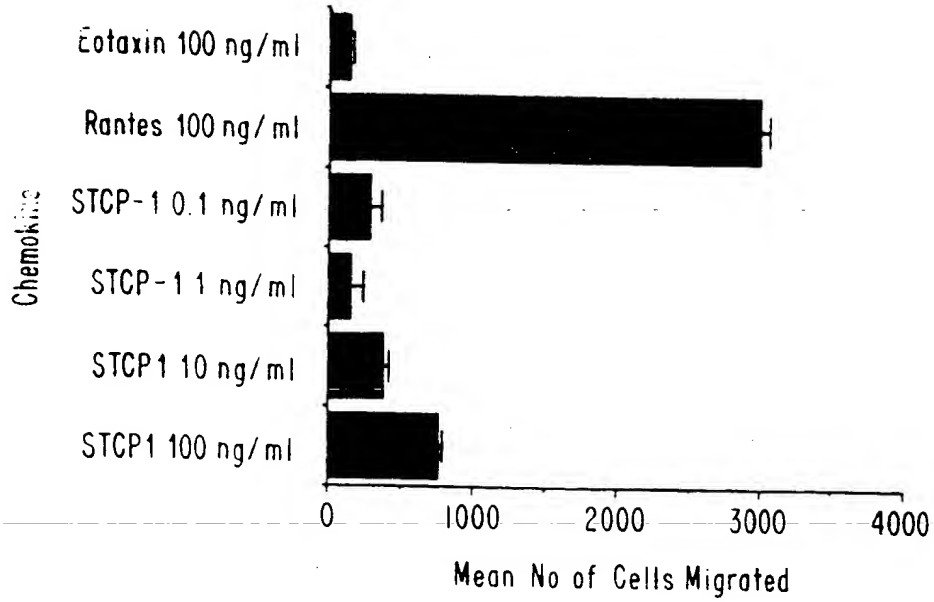

Eotaxin 100 ng/ml
Rantes 100 ng/ml
STCP-1 0.1 ng/ml
STCP-1 1 ng/ml
STCP1 10 ng/ml
STCP1 100 ng/ml
Chemokine
0
1000
2000
3000
4000
Mean No of Cells Migrated

FIG. 5B

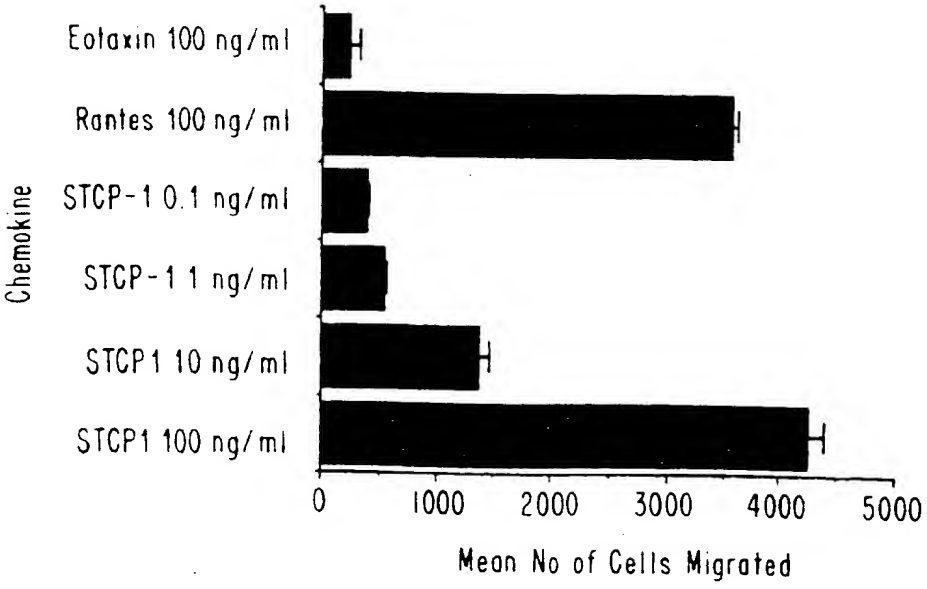

Eotaxin 100 ng/ml
Rantes 100 ng/ml
STCP-1 0.1 ng/ml
STCP-1 1 ng/ml
STCP1 10 ng/ml
STCP1 100 ng/ml
Chemokine
0
1000
2000
3000
4000
5000
Mean No of Cells Migrated

FIG. 5C

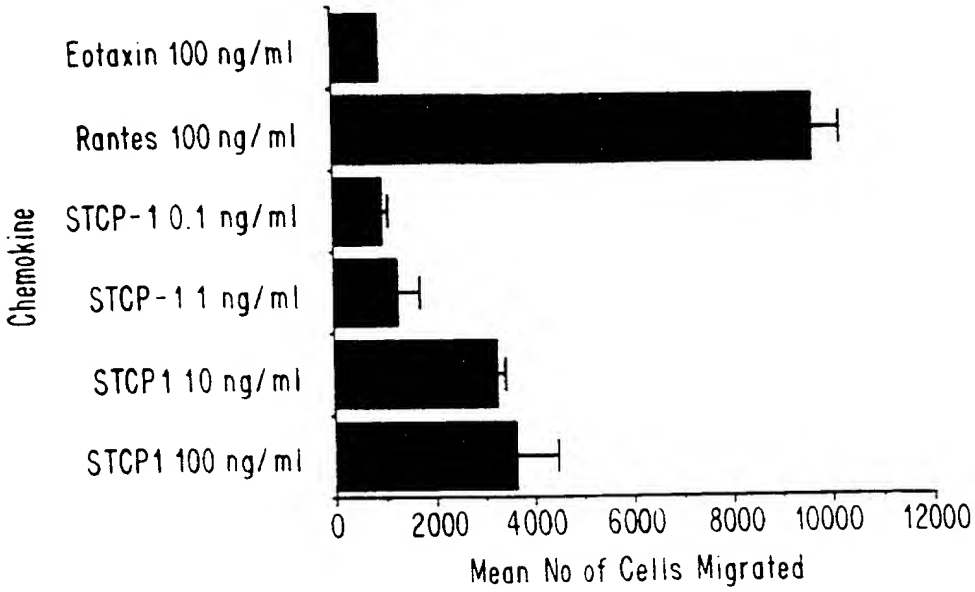



FIG. 5D

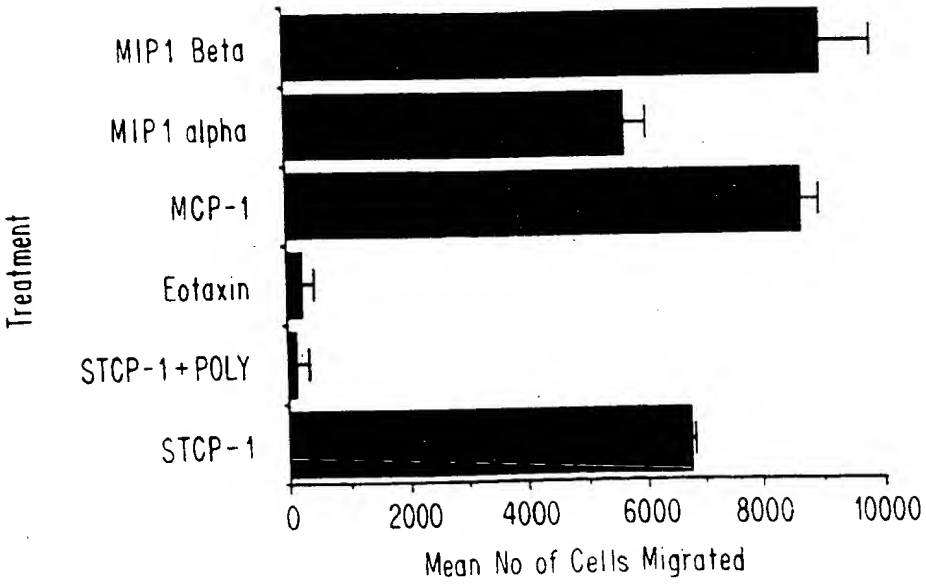


FIG.6A

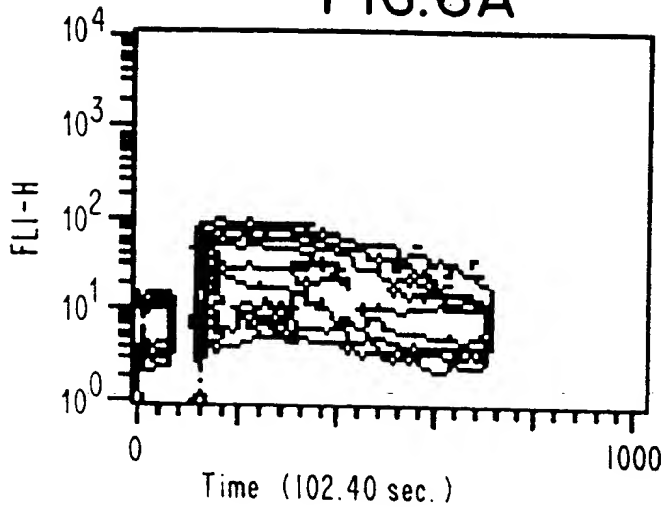



FIG.6B

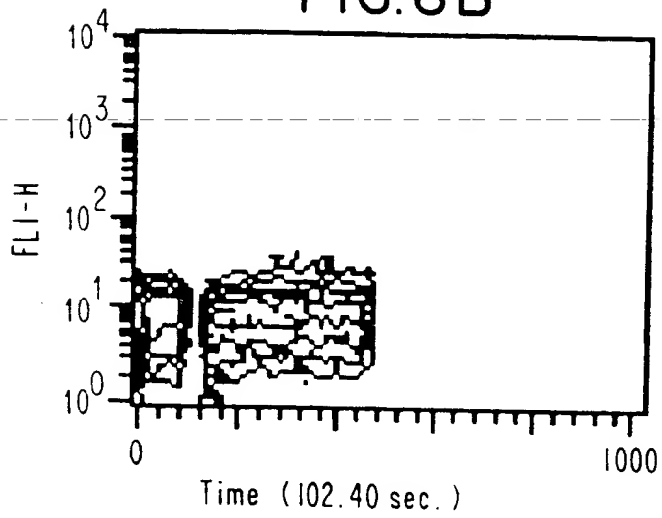



FIG.6C

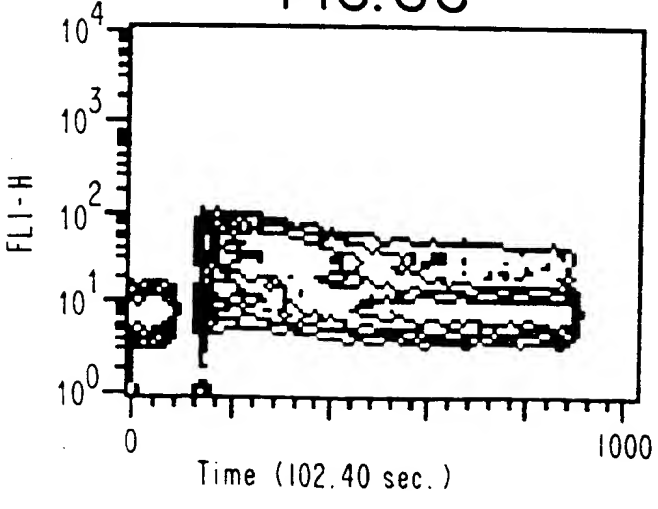


FIG. 7A

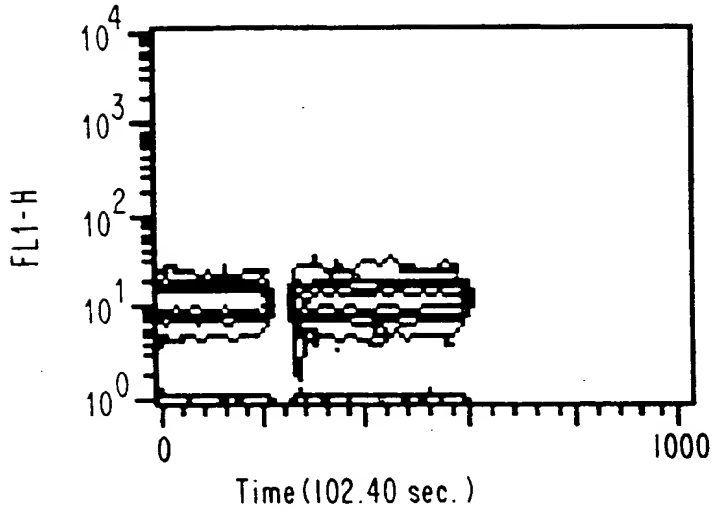



FIG. 7B

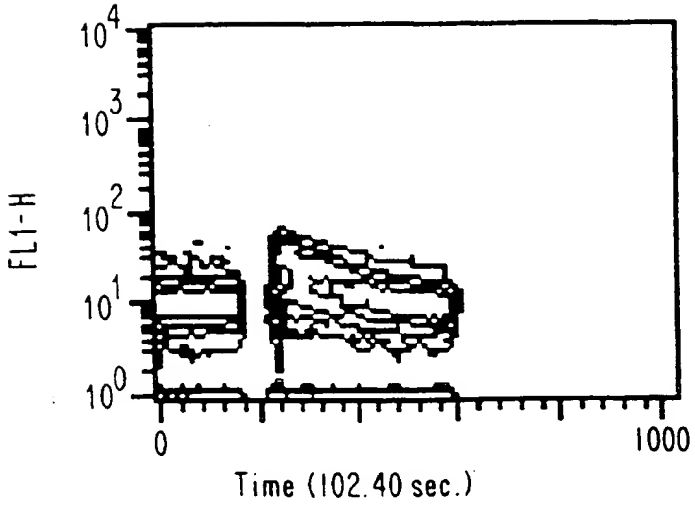


FIG. 7C

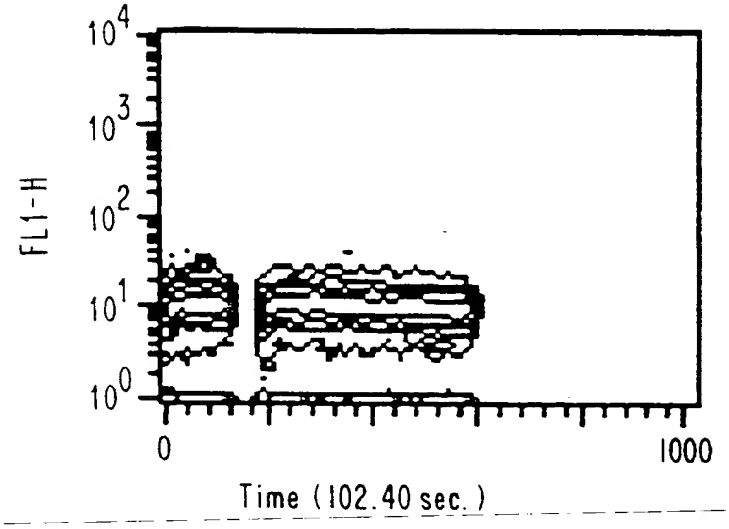



FIG. 7D

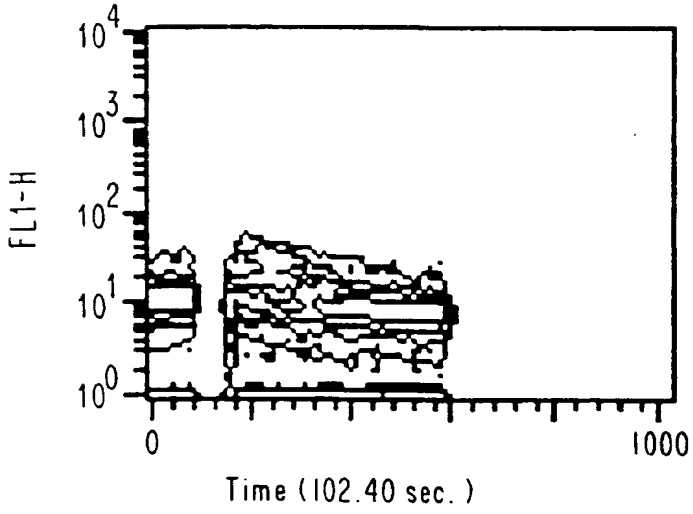
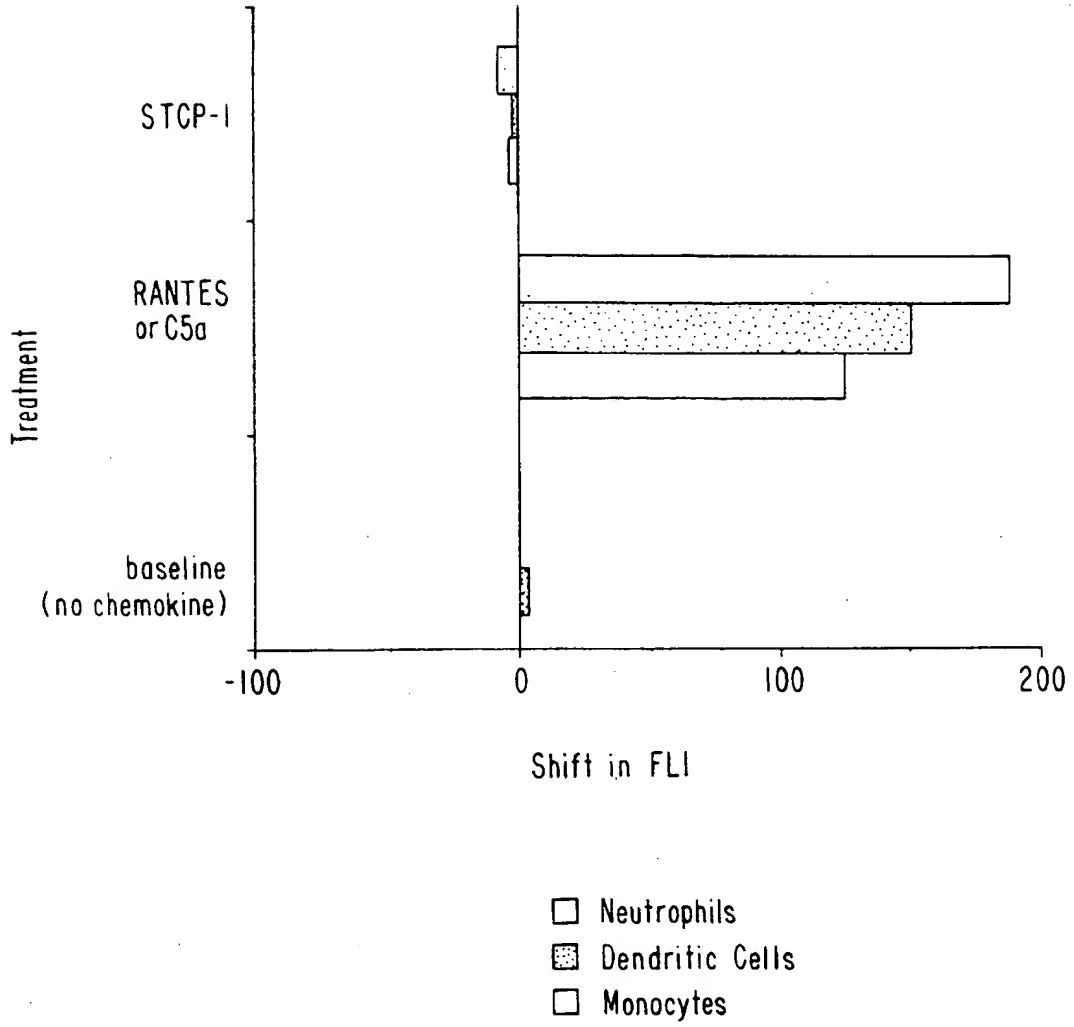


FIG.8



# INTERNATIONAL SEARCH REPORT

Int inal Application No
PCT/US 97/21552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C07K16/24 C12P21/02

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 96 39521 A (HUMAN GENOME SCIENCES INC ;SMITHKLINE BEECHAM CORP (US); LI HAODON) 12 December 1996 see page 45 - page 46 --- | 1-23 |
| P,X | WO 96 40923 A (ICOS CORP) 19 December 1996 see page 71 - page 73 see example 18 --- | 1-23 |
| E | WO 98 11226 A (SCHERING CORP) 19 March 1998 see page 76 - page 78 --- | 1-23 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of theinternational search | Date of mailing of the international search report |
|---|---|
| 15 April 1998 | 27/04/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Lejeune, R |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int. nal Application No
PCT/US 97/21552

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ³ | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | SCHALL T J ET AL: "CHEMOKINES, LEUKOCYTE TRAFFICKING, AND INFLAMMATION" CURRENT OPINION IN IMMUNOLOGY, vol. 6, no. 6, December 1994, pages 865-873, XP002048194 see the whole document | 1-23 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte nal Application No
PCT/US 97/21552

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9639521 A | 12-12-96 | AU 2820895 A | 24-12-96 |
| | | EP 0832233 A | 01-04-98 |
| WO 9640923 A | 19-12-96 | AU 6172496 A | 30-12-96 |
| | | CA 2196691 A | 19-12-96 |
| | | EP 0778892 A | 18-06-97 |
| | | FI 970502 A | 04-04-97 |
| | | HU 9701282 A | 28-10-97 |
| | | NO 970545 A | 07-04-97 |
| | | PL 318594 A | 23-06-97 |
| WO 9811226 A | 19-03-98 | NONE | |